DESIGN AND CHARACTERIZATION OF INTRANASAL BRAIN TARGETED DELIVERY OF ALMOTRIPTAN MALATE LOADED BIODEGRADABLE NANOPARTICLES FOR MIGRAINE

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI- 600 032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
BRANCH - I- PHARMACEUTICS

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APRIL 2017

CERTIFICATE

This is to certify that the M.Pharm dissertation entitled **"Design and Characterization of Intranasal Brain Targeted Delivery of Almotriptan Malate Loaded Biodegradable Nanoparticles for Migraine"** being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai was carried out by **Reg. 261510156** in the Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision, guidance and to my fullest satisfaction.

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ABBREVIATIONS

BBB	:	Blood Brain Barrier
BP	:	British Pharmacopeia
CDDS	:	Controlled Drug Delivery System
CNS	:	Controlled Nervous System
CSN	:	Chitosan Nanoparticle
DDS	:	Drug Delivery System
DSC	:	Differential Scanning Calorimetry
FTIR	:	Fourier Transform Infrared
HT	:	Hydroxy Tryptamine
IP	:	Indian Pharmacopoeia
LDC	:	Lipid Drug Conjugate
MAO	:	Monoamine Oxidase
MPS	:	Mononuclear Phagocyte System
NDDS	:	Nanoparticle Drug Delivery System
NLC	:	Nanostructured Lipid Carriers
NP	:	Nanoparticle
PBD	:	Plackett-Burman design
PEC	:	Polyelectrolyte Complex
PEG	:	Poly Ethylene Glycol
PhEur	:	European Pharmacopoeia

pI	:	Isoelectric Point
PLGA	:	Poly-D-L- lactide-co-glycolide
SLN	:	Solid Lipid Nanoparticle
SSG	:	Sodium Starch Glycolate
STPP	:	Sodium Tripolyphosphate
TEM`	:	Transmission Electron Microscopy
TGA	:	Thermogravimetric Analysis
XRD	:	X ray Diffraction Study

CONTENTS

INTRODUCTION

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The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. (R.R. Bhagwat *et. al* 2013*)*

From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition~~,~~ and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), which are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Controlled and Novel Drug Delivery which was only a dream or at best a possibility is now a reality. ~~During the last decade and half pharmaceutical and other scientists have carried out extensive and intensive investigations in this field of drug research.~~

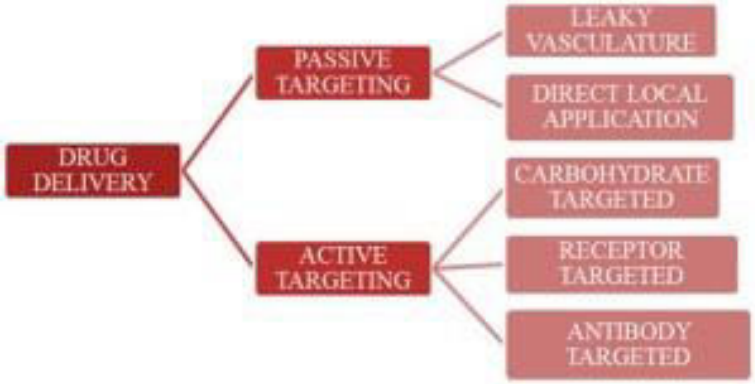
Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable, natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH- or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest. Two major mechanisms can be distinguished for addressing the desired sites for drug release:

- Passive and;

- Active targeting

An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumours as a result of the enhanced vascular permeability of tumour tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest.

Figure 1: Mechanism of drug delivery system (R.R. Bhagwat *et al* 2013)



Delivering therapeutic compound to the target site is a major problem in treatment of many diseases. A conventional application of drugs is characterized by limited effectiveness, poor bio distribution, and lack of selectivity. These limitations and drawback can be overcome by controlling drug delivery. In controlled drug delivery systems (CDDS) the drug is transported to the place of action, thus, its influence on vital tissues and undesirable side effects can be minimized. In addition, DDS protects the drug from rapid degradation or clearance and enhances drug concentration in target tissues, therefore, lower doses of drug are required. This modern form of therapy is especially important when there is a

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discrepancy between a dose or concentration of a drug and its therapeutic results or toxic effects.

Cell-specific targeting can be achieved by attaching drugs to individually designed carriers. Recent developments in nanotechnology have shown that nanoparticles (structures smaller than 100 nm in at least one dimension) have a great potential as drug carriers. Due to their small sizes, the nanostructures exhibit unique physicochemical and biological properties (e.g., an enhanced reactive area as well as an ability to cross cell and tissue barriers) that make them a favourable material for biomedical applications.

The primary goals for research of nano-bio-technologies in drug delivery include:

- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,
- Greater safety and biocompatibility, and
- Faster development of new safe medicines.

The main issues in the search for appropriate carriers as drug delivery systems are basic prerequisites for design of new materials. They comprise knowledge on

- drug incorporation and release,
- formulation stability and shelf life
- biocompatibility,
- bio distribution and targeting
- functionality.

In addition, when used solely as carrier the possible adverse effects of residual material after the drug delivery should be considered as well. In this respect, biodegradable nanoparticles with a limited life span if therapeutically needed would be optimal.

Targeted drug delivery system

The concept of designing targeted delivery system has been originated from the Paul Ehrlich, who was a microbiologist, proposed the idea of drug delivery in the form of magic bullet. Targeted drug delivery means accumulation of pharmacologically active moiety at desired target in therapeutic concentration at the same restricting its access to normal cellular lining, thus minimizing therapeutic index. The drug can be targeted to intracellular sites, virus cells, bacteria cell and parasites using different scientific strategies have proven highly effective. The minimum distribution of the parent drug to the non-target cells with higher and effective concentration at the targeted site certainly maximize the benefits of targeted drug delivery. (E. Bhargav *et.al* 2013)

Targeted delivery can be actively or passively achieved. Active targeting requires the therapeutic agent to be achieved by conjugating the therapeutic agent or carrier system to a tissue or cell-specific ligand (Lamprecht *et. al*., 2001). Passive targeting is achieved by incorporating the therapeutic agent into a macromolecule or nanoparticle that passively reaches the target organ. Drugs encapsulated in nanoparticles or drugs coupled to macromolecules can passively target tumours through the EPR effect. Alternatively, catheters can be used to infuse nanoparticles to the target organ or tissues. For example, localized delivery of drug-bearing nanoparticles to sites of vascular restenosis may be useful for providing sustained drug release at specific sites on the arterial wall (Maeda, 2001; Sahoo *et. al*., 2002).

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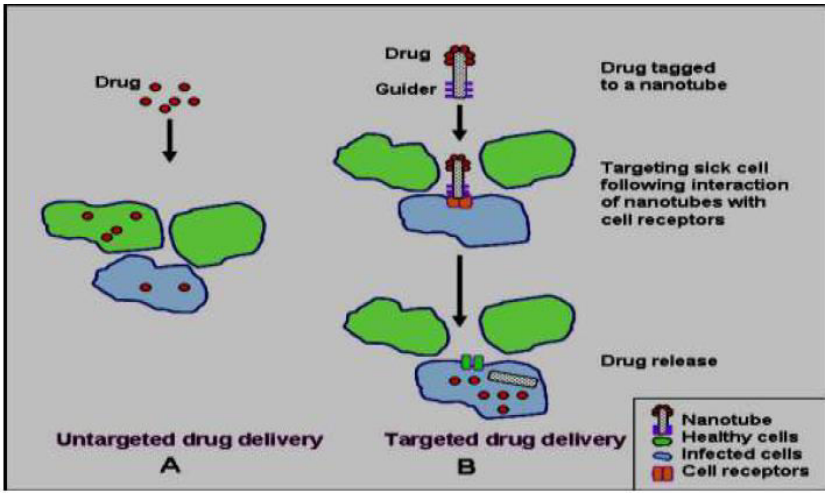
Brain targeting drug delivery system

In the central nervous system, targeted action can be achieved by direct administration of the drugs in to the CNS. Blood brain barrier can considerably

impair the effect of the large number of drugs (e.g. –antibiotics, antineoplastic agents and Neuropeptides-CNS stimulant drug) because of its obstinate hindrance affect. From some recent studies, it has been represented that the blood brain barrier is usually does not cross by almost 100% of large molecule drugs and 98% of small molecule drugs Presently, numerous approaches with enhanced pharmacodynamics effects, have been developed for the treatment of brain disorders. Drug discovery and drug delivery technologies are the two main fields where advancement is required for drug delivery to the brain. Nanoparticles drug delivery system (NDDS) is one of the advanced technology that can be utilized to deliver drug molecules directly into the brain and proved to be very effective against several CNS disorders. (Yasir Mohamed *et. al* 2015)

Figure 2: Drug Targeting Technology



Nanoparticles

Nanoparticles (NPs) containing encapsulated, dispersed, absorbed or conjugated drugs have unique characteristics that can lead to enhanced performance

in a variety of dosage forms. The aims for nanoparticle entrapment of drugs are either enhanced delivery to, or uptake by, target cells and/or a reduction in the toxicity of the free drug to non-target organs. Both situations will result in an increase of therapeutic index, the margin between the doses resulting in a therapeutic efficacy (e.g. tumour cell death) and toxicity to other organ systems. For these aims, creation of long-lived and target-specific nanoparticles is needed. For these aims, creation of long-lived and target-specific nanoparticles is needed.

When formulated correctly, drug particles are resistant to settling and can have higher saturation solubility, rapid dissolution and enhanced adhesion to biological surfaces, thereby providing rapid onset of therapeutic action and improved bioavailability. In addition, the clear majority of molecules in a nanostructure reside at the particle surface, which maximizes the loading and delivery of cargos, such as therapeutic drugs, proteins and polynucleotides, to targeted cells and tissues. Highly efficient drug delivery, based on nanomaterials, could potentially reduce the drug dose needed to achieve therapeutic benefit, which, in turn, would lower the cost and/or reduce the side effects associated with drugs. Furthermore, NP size and surface characteristics can be easily manipulated to achieve both passive and active drug targeting. Site-specific targeting can be achieved by attaching targeting ligands, such as antibodies or aptamers, to the surface of particles, or by using guidance in the form of magnetic NPs. NPs can also control and sustain release of a drug during transport to, or at, the site of localization, altering drug distribution and subsequent clearance of the drug to improve therapeutic efficacy and reduce side effects.

The key advantages of nanoparticles are

(1) improved bioavailability by enhancing aqueous solubility,

(2) increasing resistance time in the body (increasing half-life for clearance/increasing specificity for its cognate receptors

(3) targeting drug to specific location in the body (its site of action).

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This results in concomitant reduction in quantity of the drug required and dosage toxicity, enabling the safe delivery of toxic therapeutic drugs and protection of non-target tissues and cells from severe side effect. It is increasingly used in different applications, including drug carrier systems and to pass organ barriers such as the blood-brain barrier, cell membrane etc. They are based on biocompatible lipid and provide sustained effect by either diffusion or dissolution. (Wim H De Jong ~~*et al*~~*et.al* 2008)

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History of nanoparticles

The history of nanoparticles is older than the nanotechnology. The first known use of nanoparticles, made by artisans in the 9th century in Mesopotamia to create a shiny effect on the pot surfaces. Artisans Artisans created these nanoparticles by adding salts of copper and silver and oxides them with clay, vinegar and ochre, on the surface of the cover which has been made before. After creation, the object was put into a special oven and heated until it reaches about 600 °C in a decreasing atmosphere. As temperature increases in the oven, silver and copper ions slide to the outer layers of glitter.

However, Michael Faraday was the one who made the first scientific description about nanoparticles. He pointed out the optical proper ties of nanometascale metals in his classic 1857 paper.

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Use of nanoparticle in medicine and biology

In medicine and biology, nanoparticles are one of the basic things for applications. The main reason is their size and high ratio of surface area to mass. The most known applications are;

- Fluorescent biological labels,
- Drug and gene delivery
- Bio detection of pathogens
- Detection of proteins
- Probing of DNA structure
- Tissue engineering
- Tumour destruction via heating (hyperthermia) (Wim H De Jong *et al* 2008)

Drug release from nanoparticles (Sagar R. Mudshinge ~~*et al*~~*et.al* 2011)

Nanoparticles are coated with polymers that releases the drug by controlled diffusion or erosion from the core across the polymeric membrane or matrix. The membrane coating acts as a barrier to release, therefore, the solubility and diffusivity of drug in polymer membrane becomes the determining factor in drug release. The release rate is affected by ionic interaction between the drug and addition of auxiliary ingredients. When the drug is involved in interaction with auxiliary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect.

For developing a nanoparticulate system the two important factors to be considered are drug release and polymer biodegradation. Drug release rate depends on

(1) solubility of drug,
(2) desorption of the surface bound/ adsorbed drug,
(3) drug diffusion through the nanoparticle matrix
(4) nanoparticle matrix erosion/degradation and
(5) combination of erosion/diffusion process.

Thus solubility, diffusion and biodegradation of the matrix materials govern the release process.

Advantages of Nanoparticles (Yasir Mohamed ~~*et al*~~*et.al* 2015)

- Drug carrying capacity is high
- Nanoparticles releases the drug in sustained manner
- Drug Having extended time of circulation/show stability in bloodstream

Disadvantage of Nanoparticles

- Manufacturing cost is very high for this drug delivery system.
- May possibly cause allergic Reactions
- Nanoparticles may cause some toxic/unwanted reactions due to the over use of polyvinyl alcohol in their formulation

Characteristics Important for Drug Delivery using Nanoparticles

❖ Particle size

Particle size and size distribution are the most important characteristics of nanoparticles. They determine the *in vivo* distribution, biological fate, toxicity, and targeting ability of these delivery systems. In addition, they can influence drug loading, drug release, and stability of nanoparticles. Many studies have demonstrated that nanoparticles have several advantages over microparticles (Panyam and Labhasetwar, 2003). Generally, nanoparticles have relatively high cell uptake when compared to microparticles and are available to a wider range of cellular and intracellular targets due to their small size and mobility. Nanoparticles can cross the blood-brain barrier following the opening of endothelium tight junctions by hyper-osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumours (Kroll et al., 1998). Tween 80-coated nanoparticles have been shown to cross the blood-brain barrier as well (Kreuter *et al.*, 2003). Submicron nanoparticles, but not larger microparticles, are taken up by most cell types (Zauner *et al.*, 2001). Indeed, 100 nm nanoparticles had a 2.5-fold greater uptake rate than 1 µm microparticles, and a 6-fold greater uptake than 10 µm microparticles by Caco-2 cells (Desai *et al.*, 1997).

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Drug release also is affected by particle size. Smaller particles have a larger surface area-to-volume ratio; therefore, most of the drug associated with small particles would be at or near the particle surface, leading to faster drug release. In contrast, larger particles have large cores, which allow more drug to be encapsulated per particle and give slower release (Redhead *et. al.*, 2001). Thus, control of particle size provides a means of tuning drug release rates.

❖ **Surface properties of nanoparticles**

The association of a drug to conventional carriers leads to modification of the drug bio distribution profile, as it is mainly delivered to the mononuclear phagocyte system (MPS) such as liver, spleen, lungs and bone marrow. Nanoparticles can be recognized by the host immune system when intravenously administered and cleared by phagocytes from the circulation (Muller *et. al.*, 1996). Apart from the size of nanoparticles, nanoparticle hydrophobicity determines the level of blood components (e.g., opsonins) that bind this surface. Hence, hydrophobicity influences the *in vivo* fate of nanoparticles (Brigger *et. al.*, 2002; Muller *et. al.*, 1996). Indeed, once in the blood stream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the MPS (Grislain *et. al.*, 1983).

To increase the likelihood of success in drug targeting, it is necessary to minimize the opsonisation and prolong the circulation of nanoparticles *in vivo*. This can be achieved by coating nanoparticles with hydrophilic polymers/surfactants or formulating nanoparticles with biodegradable copolymers with hydrophilic characteristics, e.g., polyethylene glycol (PEG), polyethylene oxide, poloxamer, poloxamer, and polysorbate 80 (Tween 80). Studies show that PEG on nanoparticle surfaces prevents opsonization by complement and other serum factors. PEG molecules with brush-like and intermediate configurations reduced phagocytosis and complement activation, whereas surfaces comprised of PEG with mushroom-

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like structures were potent complement activators and favoured phagocytosis (Bhadra *et. -al-*, 2002; Olivier, 2005).

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles (Couvreur *et. -al-*, 2002). It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above ± 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential also can be used to determine whether a charged active material is encapsulated within the centre of the nanoparticle or on the surface.

❖ **Drug loading**

A successful Nano delivery system should have a high drug-loading capacity, thereby reducing the quantity of matrix materials for administration. Drug loading can be accomplished by two methods. The incorporation method requires the drug to be incorporated at the time of nanoparticle formulation. The adsorption/absorption methods call for absorption of the drug after nanoparticle formation; this is achieved by incubating the nano-carrier with a concentrated drug solution. Drug loading and entrapment efficiency depend on drug solubility in the excipient matrix material (solid polymer or liquid dispersion agent), which is related to the matrix composition, molecular weight, drug-polymer interactions, and the presence of end functional groups (i.e., ester or carboxyl) in either the drug or matrix (Govender *et al*et.al., 1999; Govender *et al*et.al., 2000; Panyam *et. -al-*, 2004). A polymer of choice for some nanoparticle formulations is PEG, which has little or no effect on drug-loading and interactions (Peracchia *et. -al-*, 1997). In addition, the macromolecules, drugs or protein encapsulated in nanoparticles show the greatest loading efficiency when they are loaded at or near their isoelectric point (pI) (Calvo *et al*et. al., 1997). For small molecules, studies show the use of ionic

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interaction between the drug and matrix materials can be very effective in increasing drug-loading (Chen *et. al.*, 1994; Chen *et. al.*, 2003).

❖ **Drug release**

It is important to consider both drug release and polymer biodegradation when developing a nanoparticulate delivery system. In general, the drug release rate depends on: (1) drug solubility; (2) desorption of the surface-bound or adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion or degradation; and (5) the combination of erosion and diffusion processes. Hence, solubility, diffusion, and biodegradation of the particle matrix govern the release process.

In the case of nanospheres, where the drug is uniformly distributed, drug release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than matrix erosion, then the mechanism of release is largely controlled by a diffusion process. The rapid, initial release, or 'burst', is mainly attributed to weakly bound or adsorbed drug to the relatively large surface of nanoparticles (Magenheim *et. al.*, 1993). It is evident that the method of incorporation influences the release profile. If the drug is loaded by the incorporation method, then the system has a relatively small burst effect and sustained release characteristics (Fresta *et al*et.al., 1995). If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the polymeric membrane.

Membrane coating acts as a drug release barrier; therefore, drug solubility and diffusion in or across the polymer membrane becomes a determining factor in drug release. Furthermore, the release rate also can be affected by ionic interactions between the drug and auxiliary ingredients. When the entrapped drug interacts with auxiliary ingredients, a less water soluble complex can form, which can slow the drug release – having almost no burst release effect (Chen *et al*et.al., 1994).

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Whereas if the addition of auxiliary ingredients, e.g., ethylene oxide-propylene oxide block copolymer to chitosan, reduces the interaction of the drug with the matrix material due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be achieved (Calvo et al., 1997). Various methods can be used to study the release of drug from the nanoparticle: (1) side-by-side diffusion cells with artificial or biological membranes; (2) dialysis bag diffusion; (3)—reverse dialysis bag diffusion;—(4)—agitation followed by ultracentrifugation/ centrifugation; or (5) ultra-filtration. Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred. However, these methods prove difficult to replicate and scale-up for industrial use.

TYPES OF NANOPARTICLES

Extensive libraries of nanoparticles, composed of an assortment of different sizes, shapes, and materials, and with various chemical and surface properties, have already been constructed. This field is rapidly growing and these continue to supplement these huge collections with new contributions. Some of the classes of nanoparticles are listed below

Fullerenes

A fullerene is any molecule composed entirely of carbon. These are in the form of hollow sphere, ellipsoid, or tube. Fullerenes are similar in structure to the graphite, which is composed of stacked grapheme sheets of linked hexagonal rings, additionally they may also contain pentagonal (or sometimes heptagonal) rings to give potentially porous molecules (Holister *et al*., 2003). A spherical fullerene composed of less than 300 carbon atoms are commonly known as endohedral fullerenes and include the most common fullerene. Mega tubes are larger in

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diameter than nanotubes and prepared with walls of different thickness which is potentially used for the transport of a variety of molecules of different sizes (Mitchell ~~et al~~*et al*., 2001).

Solid lipid nanoparticles

Solid lipid nanoparticles comprise of lipids that are in solid phase at room temperature and surfactants for emulsification. SLNs offer unique properties such as small size, large surface area, high drug loading, the interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, nutraceuticals and other materials. Solid lipids utilized in SLN formulations include fatty acids, triglycerin, steroids (e.g. cholesterol), partial glycerides and waxes. Several types of surfactants are commonly used as emulsifiers to stabilize lipid dispersion, including soybean lecithin, phosphatidylcholine, poloxamer 188, sodium cholate, and sodium glycocholate.. SLNs possess a better stability and ease of upgradability to production scale as compared to liposomes. This property may be very important for many modes of targeting.

Liposomes

Liposomes are vesicular structures with an aqueous core surrounded by a hydrophobic lipid bilayer, created by the extrusion of phospholipids. Solutes, such as drugs, in the core cannot pass through the hydrophobic bilayer however hydrophobic molecules can be absorbed into the bilayer, enabling the liposome to carry both hydrophilic and hydrophobic molecules. The lipid bilayer of liposomes can fuse with other bilayers such as the cell membrane, which promotes release of its contents, making them useful for drug delivery and cosmetic delivery applications.

Nanostructured lipid carriers (NLC)

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Nanostructured Lipid Carriers are produced from blend of solid and liquid lipids, but particles are in solid state at body temperature. Lipids are versatile molecules that may form differently structured solid matrices, such as the nanostructured lipid carriers (NLC) and the lipid drug conjugate nanoparticles (LDC), that have been created to improve drug loading capacity. NLCs can generally be applied where solid nanoparticles possess advantages for the delivery of drugs. Major application areas in pharmaceutics are topical drug delivery, oral and parenteral (subcutaneous or intramuscular and intravenous) route. They also have applications in cosmetics, food and agricultural products. These have been utilized in the delivery of anti-inflammatory compounds, cosmetic preparation, topical cortico therapy and also increases bioavailability and drug loading capacity.

Nano shells

Nano shells are also notorious as core-shells, Nano shells are spherical cores of a compound (concentric particles) surrounded by a shell or outer coating of thin layer of another material, which is a few 1–20 nm nanometres thick. Nano shell particles are highly functional materials show modified and improved properties than their single component counterparts or nanoparticles of the same size. Their properties can be modified by changing either the constituting materials or core-to-shell ratio.

Dendrimers

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Dendrimers are unimolecular, monodisperse, micellar nanostructures, around 20 nm in size, with a well-defined, regularly branched symmetrical structure and a high density of functional end groups at their periphery. The structure of

dendrimers consists of three distinct architectural regions as a focal moiety or a core, layers of branched repeat units emerging from the core, and functional end groups on the outer layer of repeat units. They are known to be robust, covalently fixed, three dimensional structures possessing both a solvent-filled interior core (nanoscale container) as well as a homogenous, mathematically defined, exterior surface functionality.

Quatom dots

The quantum dots are semiconductor nanocrystals and coreshell nanocrystals containing interface between different semiconductor materials. The size of quantum dots can be continuously tuned from 2 to 10 nm, which, after polymer encapsulation, generally increases to 5–20 nm in diameter. Particles smaller than 5 nm are quickly cleared by renal filtration.

Biodegradable nanoparticles

Biodegradable nanoparticles have been used for site-specific delivery of drugs, vaccines and various other biomolecules. A few of the most extensively used biodegradable polymer matrices for preparation of nanoparticles are

- **Gelatin**

Gelatin is extensively used in food and medical products and is a nontoxic alternative. Gelatin NPs are very efficient in delivery and controlled release of the drugs. They are nontoxic, biodegradable, bioactive and inexpensive. Gelatin is a poly-ampholyte consisting of both cationic and anionic groups along with a hydrophilic group. It is known that the mechanical properties such as swelling

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behavior and thermal properties of gelatin NPs depend significantly on the degree of cross-linking between cationic and anionic groups. These properties of gelatin can be manipulated to prepare desired type of NPs from gelatin. Gelatin nanoparticles can be prepared by the desolvation/coacervation or emulsion methods.

- **Chitosan**

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Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of the crustacean-derived natural biopolymer chitin. There are at least four methods reported for the preparation of chitosan nanoparticles. The four methods are ionotropic gelation, micro emulsion, emulsification solvent diffusion and polyelectrolyte complex formation.

- **Poly-D-L- lactide-co-glycolide (PLGA)**

Poly-D-L- lactide-co-glycolide (PLGA) is one of the most successfully used biodegradable polymers. It undergoes hydrolysis in the body to produce biodegradable metabolite monomers such as lactic acid and glycolic acid. Since lactic acid and glycolic acids are normally found in the body and participate in several physiological and biochemical pathways, there is very minimal systemic toxicity associated with the use of PLGA for the drug delivery or biomaterial applications. PLGA NPs have been mostly prepared by the emulsification-diffusion, the solvent evaporation and the nanoprecipitation methods . PLGA nanoparticles have been used to develop protein and peptide based nanomedicines, nano-vaccines, and genes containing nanoparticles for in-vivo delivery systems.

- **Polylactic acid (PLA)**

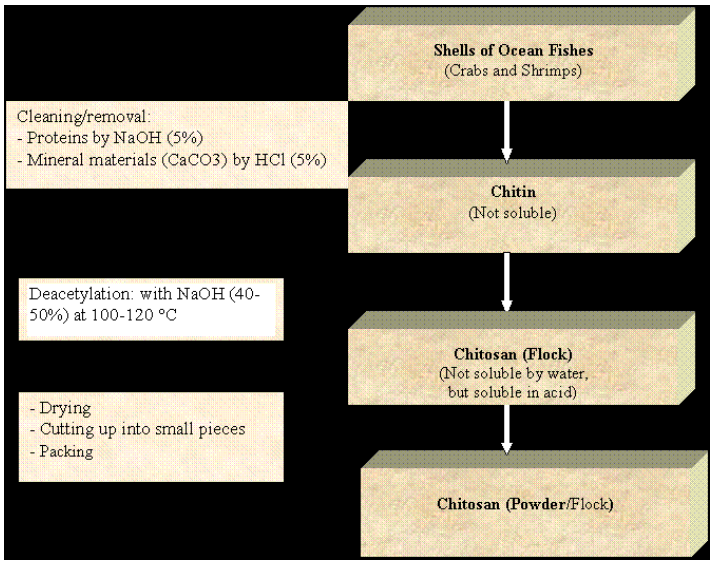
PLA is a biocompatible and biodegradable polymer which is broken down to monomeric units of lactic acid in the body. Lactic acid is a natural

intermediate/by product of anaerobic respiration, which is converted into glucose by the liver during the Cori cycle. Glucose then is used as an energy source in the body. The use of PLA nanoparticles is therefore safe and devoid of any major toxicity. PLA nanoparticles have been mostly prepared by the solvent evaporation, solvent displacement, salting out and solvent diffusion methods. The salting out procedure is based on the separation of a water- miscible solvent from aqueous solution by adding a salting out agent like magnesium chloride or calcium chloride. The main advantage of the salting out procedure is that it minimizes stress to protein encapsulants.

CHITOSAN NANOPARTICLES

Chitosan is a natural polymer obtained by deacetylation of chitin from crustacean shells such as crabs, shrimps and lobsters. After cellulose chitin is the second most abundant polysaccharide in nature. It is biologically safe, non-toxic, biocompatible and biodegradable polysaccharide.

Figure 3: chitosan extraction



Chitosan is a biodegradable, biocompatible, positively charged nontoxic Mucoadhesive biopolymer. Since chitosan contains primary amino groups in the main backbone that make the surfaces positively charged in biological fluids, biodegradable nano/microparticles can be readily prepared by treating chitosan with a variety of biocompatible polyanionic substances such as sulphate, citrate, and tripolyphosphate.

Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method and providing versatile routes of administration. Chitosan has the special possibility of adhering to the mucosal surfaces with in the body because of their sub-micron size and is suitable for mucosal routes of administration i.e. oral, nasal and ocular mucosa which is non-invasive route. Chitosan nanoparticles showed to be a good adjuvant for vaccine also.

Advantages of chitosan nanoparticles

- Simple and inexpensive to manufacture and scale-up
- No heat, high shear forces or organic solvents involved in their preparation process
- Reproducible and stable
- Applicable to a broad category of drugs; small molecules, proteins and polynucleotides
- Ability to lyophilize
- Stable after administration
- Non-toxic

PREPARATION METHODS OF CHITOSAN NANOPARTICLES

Ionotropic gelation

Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo et al., (1997b) and has been widely examined and developed (Janes *et al*., 2001; Pan *et al*., 2002). The mechanism of chitosan NP formation is based on electrostatic interaction between amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate (Bodmeier *et al*., 1989; Xu and Du, 2003). This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers

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was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer.

Micro emulsion method

Chitosan NP prepared by micro emulsion technique was first developed by Maitra et al. This technique is based on formation of chitosan NP in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in N-hexane. Then, chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant. The system was stirred overnight to complete the cross- linking process which the free amine group of chitosan conjugates with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with $CaCl_2$ and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before lyophilisation. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step.

Emulsification solvent diffusion method

NP prepared by emulsion solvent diffusion method, (El-Shabouri, 2002) which originally developed by Niwa et al. employing PLGA. This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection an organic phase into chitosan solution containing a stabilizing agent (i.e. poloxamer) under mechanical stirring, follow by high pressure

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homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs because of the diffusion of organic solvent into water, leading to the formation of nanoparticles.

Polyelectrolyte complex (PEC)

Polyelectrolyte complex or self-assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA. Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self-assembly. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature (Erbacher *et al*., 1998). The complexes size can be varied from 50 nm to 700nm.

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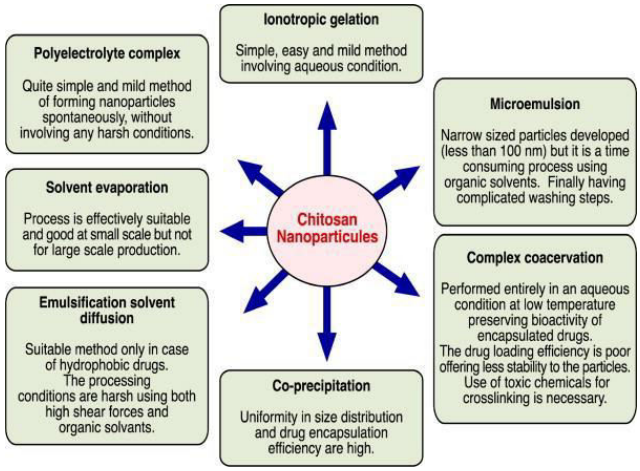
Figure 4: Preparation methods of chitosan nanoparticles



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Nasal drug delivery system

In recent years, the nasal route has received a great deal of attention as a convenient and reliable method for the systemic administration of drugs. However, polar drugs and some macromolecules are not absorbed in sufficient concentration due to poor membrane permeability, rapid clearance and enzymatic degradation into the nasal cavity. Earlier studies have demonstrated that intranasal administration offers a practical, non-invasive and an alternative route of administration for rapid drug delivery to brain. The large surface area of the nasal cavity and the relatively high blood flow, thereby achieving a rapid absorption and avoidance of hepatic first-pass elimination are attractive features of nasal drug administration. It also offers the advantages of being administered simply, cost effectively and conveniently.

Diseases of the Central Nervous System (CNS) such as schizophrenia, meningitis, migraine, Parkinson's disease and Alzheimer's disease require delivery

of the drug to the brain for treatment. However, such transport remains problematic, especially for hydrophilic drugs and large molecular weight drugs, due to the impervious nature of the endothelial membrane separating the systemic circulation and central interstitial fluid, the Blood–Brain Barrier (BBB). Some of the currently employed invasive approaches (mechanically breaching the BBB) include interstitial delivery, intracerebroventricular delivery, intracerebral delivery, and convection enhanced delivery. It has been shown transport of exogenous materials directly from nose-to-brain is a potential route for by-passing the BBB. This route, involves the olfactory or trigeminal nerve systems which initiate in the brain and terminate in the nasal cavity at the olfactory neuroepithelium or respiratory epithelium, respectively.

Advantages of Nasal Drug Delivery

1) Drug degradation that is observed in the gastrointestinal track is absent.
2) Hepatic first pass metabolism is avoided.
3) The nasal bioavailability for smaller drug molecule is good.
4) Studies so far indicates that the nasal route is an alternative to parenteral route, especially for protein's and peptide drug.
5) Convenient for the patient especially for those on long term therapy, when compared with parenteral medication.
6) Polar compound exhibiting poor oral absorption may be particularly studies for this route of delivery.
7) Large nasal mucosa surface area for dose absorption.
8) Ease of administration, non-invasive.
9) Lower dose reduced side effects.
10) Self-administration.

Limitations

1) Delivery is expected to decrease with increasing molecular weight of drug.
2) Mucosal damages may occur due to frequently use of intra nasal route.
3) Difficult to administered drug in pathological condition such as nasal congestion due to cold or allergic reaction.
4) Some drug cannot have administered through this route because they cause nasal irritation

MIGRAINE

Migraine is a syndrome that affects a significant fraction of the world population, with a higher prevalence in women (15%) than in men (6%). Migraine is characterized by an intense and throbbing headache associated with anorexia, nausea, vomiting, photophobia, phonophobia which is also accompanied by impairment of cognitive and motor functions. Migraine is more than "just a headache". It is a complex neurological condition, which can affect the whole body and can result in many symptoms, sometimes without a headache at all. It can be easily overlooked or mistaken for other conditions and can affect people in different ways. Research is continuing, but at present we do not know what causes migraine; there is no clear diagnostic test and, yet, there is no cure. However, there are many ways to help manage the condition and lessen its impact – ultimately reducing the disruption caused to everyday life.

Symptoms

Symptoms of migraine can occur a while before the headache, immediately before the headache, during the headache and after the headache. Although not all migraines are the same, typical symptoms include:

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- ✓ Moderate to severe pain, usually confined to one side of the head during an attack, but it can occur on either side of the head
- ✓ The pain is usually a severe, throbbing, pulsing pain
- ✓ Increasing pain during physical activity
- ✓ Inability to perform regular activities due to pain
- ✓ Feeling sick and physically being sick
- ✓ Increased sensitivity to light and sound, relieved by lying quietly in a darkened room
- ✓ Some people experience other symptoms such as sweating, temperature changes, tummy ache, nausea or vomiting and diarrhoea.
- ✓ Neurological symptoms that include visual disturbances such as blind spots, distorted vision, flashing lights or zigzag patterns;
- ✓ Other common aura symptoms you may experience include: tingling or pins and needles in the limbs, an inability to concentrate, confusion, difficulty in speaking, paralysis or loss of consciousness (in very rare cases).

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The five stages of an attack

Although not all migraines follow the same pattern, there generally tend to be Five phases of a migraine attack:

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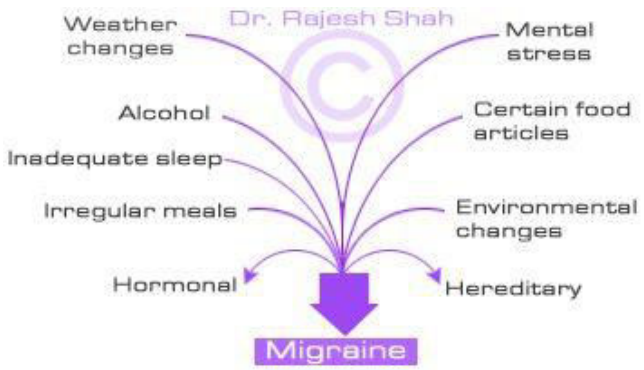
- The prodrome (warning) stage: Signs, such as mood changes, tiredness, an unusual hunger or thirst can happen up to 48 hours before an attack.
- The aura: This part of the attack can last up to an hour and usually precedes the headache. Symptoms may include visual disturbances, pins and needles, confusion etc.

- The main stage of the attack: A headache will often be present along with other symptoms, such as nausea and / or vomiting and can last between 4 and 72 hours.
- Resolution / postdrome stage: The pain gradually eases or may disappear, but feelings of lethargy or being 'washed-out' may remain.
- Recovery stage: It can take a few days to fully recover, or for the more lucky ones, recovery can be surprisingly quick.

Common migraine 'triggers'

- Stress
- Lack of food or infrequent meals
- Certain foods including products of caffeine, tyramine, alcohol, monosodium glutamate
- Changing sleep patterns
- Hormonal patterns e.g. monthly periods, contraceptive pills.
- Extreme emotions e.g. anger, or grief
- Environmental factors e.g. loud noise, bright, flickering lights, strong smells, hot stuffy atmospheres
- Climatic conditions e.g. strong winds, extreme heat or cold.

Figure 5: Migraine Triggers



Migraine Treatment : Multifactorial Causes

Pathophysiology of migraine

Pathogenesis of migraine involves not only the intra and extra-cranial vasodilation but also activation of pain receptors of trigeminovascular system.

In many cases, migraine attacks are likely to begin centrally, in brain areas capable of generating the classical neurological symptoms of prodromes and aura, whereas the headache phase begins with consequential activation of meningeal nociceptors at the origin of the trigeminovascular system (Noseda and Burstein, 2013). While some clues about how the occurrence of aura can activate nociceptors in the meninges exist, nothing is known about the mechanisms by which common prodromes initiate the headache phase or what sequence of events they trigger that results in activation of the meningeal nociceptors. A mechanistic search for a common denominator in migraine symptomatology and characteristics points heavily toward a genetic predisposition to generalized neuronal hyperexcitability

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(Ferrari et al., 2015). Mounting evidence for alterations in brain structure and function that are secondary to the repetitive state of headache can explain the progression of disease (Sprenger and Borsook, 2012).

The complexity of pathophysiological mechanisms involved in migraine impedes its treatment, although a range of drugs with diverse pharmacological activities have evolved for its therapeutic management.

The $5HT_{1B}$ receptor located in large numbers on smooth muscle of meningeal blood vessels that mediates vasoconstriction; they are ideally placed to reverse the meningeal vasodilation that occurs during migraine attack. Triptans are warranted, because triptans are selective $5HT_{1B/1D}$ agonists; and most effective in aborting headache when used early in pain phase.

Migraine treatments

Initially, migraine-specific management was based on the vascular model of headache advanced by Wolff and Graham after their seminal article in 1938 linking migraine to vasoconstriction; thus, vasoconstriction agents were sought that could terminate an attack. As a result, ergot preparations were developed and were efficacious, but with significant side effects. The 1990s saw a major advance with the introduction of Sumatriptan. This serotonin receptor 5-hydroxytryptamine 1 (5-HT_1) agonist was as effective as ergots but generally produced fewer concerning side effects. Presently, there are seven triptans. Their exact mechanism of action in migraine is unclear. They may inhibit neurogenic inflammation peripherally, inhibit nociceptive inputs to the central pain system, or act as peripheral vasoconstrictors. Further migraine-specific agents are in development.

Table 1: Drugs for treatment of migraine

Drug	Dose	~~Half life~~Half-life in hours	Maximum daily dose
Group 1: Fast Onset Triptans			
Almotriptan	6.25mg and 12.5 mg	3-4 hours	25 mg
Elitriptan	20 mg and 40 mg	4 hours	80mg
Rizatriptan	5mg and 10 mg	2-3 hours	30mg
Sumatriptan	25mg, 50 mg and 100mg- orally	3 hours	200mg- oral
	5mg and 20mg – intra nasal		40mg – intra nasal
	6mg/0.5ml - subcutaneous		12mg - subcutaneous
Zolmitriptan	2.5mg and 5mg	3 hours	10mg
Group 2: slower onset triptans			
Frovatriptan	2.5mg tablet	26	7.5
Naratriptan	1mg and 2.5 mg	6	5

RESEARCH OBJECTIVE

The rationale of this study is to design and evaluate the characteristics of Intra nasal delivery of chitosan nanoparticles containing almotriptan malate to treat migraine. In the present research work an attempt was made to develop a novel dosage form of intranasal brain targeted nanoparticles of almotriptan malate by using a biodegradable polymer chitosan using ionic gelation technique.

Almotriptan malate was selected as a model drug because of the following reasons:

- It is used in treatment of migraine
- It is having better water soluble property
- It is having a shorter biological half-life of 4 hours

OBJECTIVE OF THE STUDY

- To synchronise nasal drug delivery for enhanced activity
- To enhance brain targeting of hydrophilic drug
- To reduce the dose of drug.
- To reduce dose dependent side effect.
- To develop a sustained release formulation.
- To overcome drug resistance.

PLAN OF RESEARCH WORK

- Literature survey on almotriptan malate, nanoparticles, chitosan nanoparticles, migraine
- Selection of polymers
- Preformulation studies
- Preparation of chitosan nanoparticles loaded with almotriptan malate
- Stastical optimisation
- Characterisation studies and invitro drug release studies
- Stability studies

LITERATURE REVIEW

1. **Girotra P *et al.*, (2016)** developed Poly (D, L Lactide-co-Glycolide) poloxamer nanoparticles (NPs) of the hydrophilic drug Zolmitriptan using quality-by-design approach for brain targeting. Randomized 2^4 full factorial design was employed to achieve the critical quality attributes of minimized particle size and maximized encapsulation efficiency. The PLGA/poloxamer NPs were fabricated using modified double emulsion solvent diffusion technique. The optimized nanoparticles were characterized by FTIR spectroscopy and powder X-ray diffraction technique which indicated the loading of drug in NPs without any chemical interactions between drug and the excipients. The uniform and spherical shape of the particles was affirmed from TEM analysis. The in-vivo studies for determination of brain uptake potential demonstrated the pharmacodynamic studies involving Swiss albino mice further confirmed successful delivery of drug to brain circumventing the blood brain barrier, through significantly enhanced anti-migraine potential.

2. **Girotra P *et al.*, (2016)** developed a brain-targeted rizatriptan benzoate-loaded solid lipid nanoparticles (RB-SLNs) by design of experiment, for improvement of its anti-migraine potential. Several formulation variables affecting the fabrication of RB-SLNs were screened using the Plackett-Burman design (PBD). The PBD results demonstrated lipid (Precirol® ATO 5) concentration, co-surfactant (Phospholipon® 90 H) concentration and temperature of lipid melt to be the critical variables, having a significant effect on the achievement of minimum particle size, maximum entrapment efficiency coupled with sustained drug release. The optimized formulation was, thereafter, characterized by FTIR spectroscopy, wide angle XRD, thermal analysis and TEM imaging technique. The in vivo

studies revealed the brain uptake potential of optimized RB-SLNs to be 18.43-folds higher the significant anti-migraine efficacy of RB-SLNs was corroborated through the pharmacodynamic studies on adult male Swiss albino mice. The results hence explicate that RB-SLNs have distinctly improved brain target ability and offer an apt approach for the efficient therapeutic management of migraine

3. **Sirisha Yella *et al.,* (2016).,** prepared and determined the effect of various diluents and super disintegrants in various ratios on drug release of Almotriptan. Orally disintegrating tablets of almotriptan have been developed to increase the bioavailability of the drug using optimization technique and effect of various excipients have been studied to obtain an optimized formulation. Diluents, microcrystalline cellulose, spray dried lactose, mannitol and starch were employed and superdisintegrants, cross povidone, cross carmellose sodium & sodium starch glycolate were used in different ratios. All the tablets were evaluated for precompression and post compression parameters. All the precompression parameters were found to be satisfactory. Among all the formulations F23having mannitol as diluent & SSG (8%) as superdisintegrant has shown maximum drug release of 100.03% within 20min whereas the marketed formulation shows release of 89.97%. The drug release from the optimised formulation followed first order kinetics.

4. **Sunena *et al.,* (2016)** formulated thiolated chitosan nanoparticles of zolmitriptan for brain targeting intranasal drug delivery. The thiolated chitosan was synthesized by reaction of thiogycolic acid and chitosan in presence of catalyst. The thiolated chitosan was characterized using FTIR and thermal studies. Drug loaded nanoparticles were prepared by ionic gelation of thiolated chitosan by using sodium tripolyphosphate. The

prepared nanoparticles were characterized by FTIR spectroscopy to confirm the drug loading without any chemical interactions between the drug and excipients. The morphology and size of the nanoparticles were affirmed by using TEM analysis. The drug permeation of nanoparticles through nasal mucosa was evaluated by Franz diffusion cell for intranasal drug delivery.

5. **Girotra Priti Hansraj *et al.,* (2015)** developed chitosan solid lipid nanoparticles (SLN), containing sumatriptan succinate using solvent injection method and to optimize the formulations for brain targeting potential. The formulation optimization was performed using three factor two level full factorial design the particle size, zeta potential and entrapment efficiency for all the batches were in the range of 192–301.4 nm, 30.2–51.4 mV and 76.3–91.1% respectively. The optimized formulation showed a 4.54-fold increase in brain/blood ratio of drug after 2 h of drug administration in male Wistar rats. The optimized nanoparticles were characterized by FT-IR spectroscopy, DSC, TGA, powder X-ray diffraction study and TEM analysis. The optimized formulated SLN exhibited sustained in vitro drug release by anomalous transport mechanism.

6. **Hansraj GP *et al*., (2015)** prepared chitosan solid lipid nanoparticles (SLN), containing sumatriptan succinate using solvent injection method and optimized the formulations for brain targeting potential. The formulation optimization was performed using three factor two level full factorial design. It minimizes the particle size and zeta potential, maximize the entrapment efficiency as well as maximize the concentration of drug in brain with maximized brain/plasma ratio of the drug. The optimized nanoparticles were characterized by FT-IR spectroscopy, DSC, TGA,

powder X-ray diffraction study and TEM analysis. The hydrophilic drug sumatriptan succinate, loaded in chitosan SLN, can be successfully targeted to brain via oral delivery and thus present an effective approach for the therapeutic management of migraine.

7. **Bhanushali *et al.*, (2014)** developed intranasal Nano emulsion and gel formulations for rizatriptan benzoate for prolonged action. Nano emulsion formulations were prepared by constructing pseudo-ternary phase diagrams using lipophilic and hydrophilic surfactants and water. Various Mucoadhesive agents were tried out to form thermo-triggered Mucoadhesive Nano emulsions. Mucoadhesive gel formulations of rizatriptan were prepared using different ratios of HPMC and Carbopol 980. Comparative evaluation of intranasal Nano emulsions and intranasal Mucoadhesive gels indicated that greater brain-targeting could be achieved with Nano emulsions.

8. **Patil Satish K *et al.*, (2014)** developed a new type of solid lipid nanoparticles (SLN) of naratriptan by incorporating tripalmitine containing oils in the solid core of said particle. The hot high pressure homogenization technique was used as production methods for SLNs. Naratriptan is provide nose-to-brain delivery for brain targeting and sustained release. The drug-excipient study was performed by DSC and FTIR. The Solid Lipid Nanoparticles was evaluated and optimized for various parameters such as particle size, polydispersity index, zeta potential, encapsulation efficiency, in vitro drug release and stability studies were found to be110±1.4nm, 0.22±0.02, –16.21±4.02, 95.20±0.1 and 98 % drug release respectively. SLNs formulation was subjected to stability study over a period of 3 month

9. **Karva G. S *et al.*, (2013)** developed a quantitative estimation of Almotriptan Malate in pharmaceutical formulation. The stock solution of Almotriptan Malate was prepared and subsequent suitable dilution was prepared in 0.1N HCl to obtained standard curve. The standard solution of Almotriptan Malate showed two absorption maxima, one at 283.000 nm and another at 283.00 nm. The drug obeyed beer lambert's law in the concentration range of 10-100 µg/ mL with regression 0.9998 at 283.00 nm. The results of analysis have been validated as per ICH guidelines. The developed method can be adopted in routine analysis of Almotriptan Malate in tablet dosage form as well bulk dosage form.

10. **Narayana Charyulu R *et al.*, (2013)** designed and evaluate melt in mouth films of almotriptan using polymers pullulan, carragennan, xanthan gum and guar gum as the film forming agents. Glycerol was incorporated as plasticizer to improve flexibility of films, Sorbitol as sweetener and Sodium starch glycolate as disintegrant. This preparation melt in mouth films of almotriptan with the purpose of developing a dosage form for quick onset of action, which will be beneficial in managing severe condition of migraine attack, aiding in enhancement of bioavailability and easy for administration. The films were prepared by solvent casting method. They were evaluated for physicochemical characterization such as uniformity of weight, thickness, folding endurance, uniformity of drug content, surface pH, percentage elongation and tensile strength all of which showed satisfactory results. The formulations were also subjected for in vitro disintegration and in vitro drug release.

11. **Neha Gulati *et al.*, (2013)** developed and evaluated sumatriptan succinate-loaded chitosan nanoparticles for. Sumatriptan succinate-loaded chitosan nanoparticles were successfully formulated via the ionotropic gelation

technique using the Taguchi design for optimization. The obtained nanoparticles easily penetrate the nasal mucosa by particle size. The formulation displayed sustained release up to 24 hours which may help to reduce multiple daily doses to once per day.

12. **Sagar S. Jadhav and Aparna V. Bhalerao., (2013) formulated** and evaluated intranasal Mucoadhesive nanoparticles of Rizatriptan benzoate (RZB) for treatment of Migraine. RZB loaded Chitosan (CS) nanoparticles were prepared by ionic gelation of CS with tripolyphosphate anions (TPP). The maximum entrapment efficiency and drug content was 69.1% and 60.63%, shown by optimized formulation. Particle size of optimized formulation was 0.248 µ shown by Zetasizer. Spray drying of optimized formulation was carried out and process yield was determined, which was found to be 38.78%. Spray dried nanoparticles were evaluated by DSC, X-ray diffraction pattern to study crystalline/amorphous nature of nanoparticles. The percentage mucoadhesion on nasal mucosa of goat was found to be 29.4%. The release behaviour of CS nanoparticles were evaluated in phosphate buffer pH 6.5, revealed that RZB loaded CS nanoparticles is most suitable for intranasal drug delivery.

13. **Saugat Adhikari *et al.*, (2012)** formulated chitosan biodegradable nanoparticles of zolmitriptan for migraine treatment by the ionic gelation method using polyanion sodium tripolyphosphate (STPP) as a cross-linking agent and their in-vitro characteristics were studied. Solid state analysis was undertaken using thermal methods (DSC/MDSC). The particle size and morphology was determined by laser scattering technique and transmission electron microscopy, respectively. FTIR and DSC showed no significant interactions between chitosan and drug, STPP and drug. The prepared nanoparticle shows maximum entrapment efficiency

with narrow particle size range. In dissolution testing zolmitriptan chitosan nanoparticle showed immediate release of ZMT followed by sustained release.

14. **Akhila Alladi *et al.,* (2012*)*** formulated and evaluated taste masked Almotriptan orally disintegrating tablet by using different taste masking agents and different superdisintegrants in different ratios. Two taste masking agents namely Eudragit EPO and Precirol A To5 are used to taste mask the drug. The oral disintegrating tablets of Almotriptan were prepared using different superdisintegrants and the effect of different superdisintegrants at different concentration on in-vitro release was studied. Almotriptan release from ODT was directly proportional to the concentration of the superdisintegrant used. The optimized formulation was found to release the drug in minimum time and is found to be stable**.**

15. **Bhavna, V. Sharma *et al.*, (2007)** prepared and characterised chitosan nanoparticles for nose to brain delivery of a cholinesterase inhibitor. he cholinesterase inhibitor chitosan nanoparticles were prepared by ion gelation method and characterized for the particle size, morphology and particle size distribution. The chitosan nanoparticles had a particle diameter ranging from 100-200 nm and the shape was spherical the nanoparticles showed a loading efficiency up to 92% and a loading capacity up to 50% (w/w). The Mucoadhesive property of chitosan and ability of Tween 80 to cross BBB will provide effective delivery of cholinesterase inhibitors from nose-to-brain to cross BBB. Thus, chitosan nanoparticles possess a potential to deliver cholinesterase inhibitor through the nasal mucosa to reach the brain for the treatment of neurodegenerative disease.

DRUG PROFILE

ALMOTRIPTAN MALATE

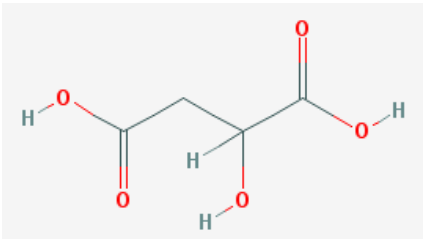
Chemical formula

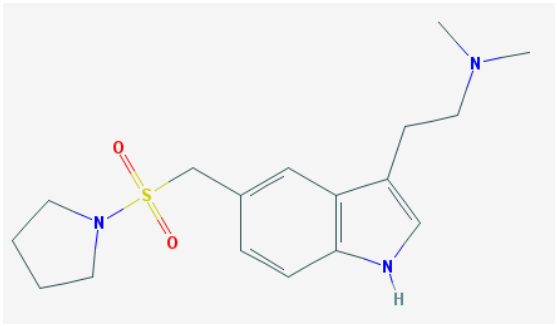
$C_{21}H_{31}N_3O_7S$

Chemical name

1-(((3-(2-(Dimethylamino)ethyl)indol-5-yl)methyl)sulfonyl)pyrrolidine.

Chemical structure





Physical state

White to slightly yellow crystalline powder

Solubility

Freely soluble in water

Molecular weight

469.553 g/mol

Drug category

Selective serotonin receptor agonists

Dosage forms

6 and 12.5mg oral tablets

Mechanism of action

Almotriptan binds with high affinity to 5-HT1D, 5-HT1B, and 5-HT1F receptors. Almotriptan has weak affinity for 5-HT1A and 5-HT7 receptors, but has no significant affinity or pharmacological activity at 5-HT2, 5-HT3, 5-HT4, 5-HT6; alpha or beta adrenergic; adenosine (A1, A2); angiotensin (AT1, AT2); dopamine (D1, D2); endothelin (ETA, ETB); or tachykinin (NK1, NK2, NK3) binding sites.

Current theories on the etiology of migraine headache suggest that symptoms are due to local cranial vasodilatation and/or to the release of vasoactive and pro-inflammatory peptides from sensory nerve endings in an activated trigeminal system. The therapeutic activity of almotriptan in migraine can most likely be attributed to agonist effects at 5-HT1B/1D receptors on the extra cerebral, intracranial blood vessels that become dilated during a migraine attack, and on nerve terminals in the trigeminal system. Activation of these receptors results in cranial vessel constriction, inhibition of neuropeptide release, and reduced transmission in trigeminal pain pathways.

Pharmacokinetic data

Absorption

The compound has a half-life of approximately 3 hours. Almotriptan is well absorbed after oral administration and the mean absolute bioavailability is 69.1%. Maximal plasma concentrations are achieved between 1.5 and 4 hours after dose administration; however, within 1 hour after administration, plasma concentrations are approximately 68% of the value at 3 hours after administration.

Distribution and protein binding

Almotriptan is not highly bound to plasma proteins and the mean unbound fraction is >60%. Therefore, plasma protein binding is not a major factor affecting the pharmacokinetics of almotriptan. The mean apparent volume of distribution is approximately 180 to 200 litres. The large volume of distribution and low degree of plasma protein binding are properties.

Metabolism

Almotriptan is metabolized by one minor and two major pathways. Monoamine oxidase (MAO)-mediated oxidative deamination (approximately 27% of the dose), and cytochrome P450-mediated oxidation (approximately 12% of the dose) are the major routes of metabolism, while flavin monooxygenase is the minor route. MAO-A is responsible for the formation of the indoleacetic acid metabolite, whereas cytochrome P450 (3A4 and 2D6) catalyses the hydroxylation of the pyrrolidine ring to an intermediate that is further oxidized by aldehyde dehydrogenase to the gamma-aminobutyric acid derivative. Both metabolites are inactive.

Excretion

Approximately 40% of an administered dose is excreted unchanged in urine. Renal clearance exceeds the glomerular filtration rate by approximately 3-fold, indicating an active mechanism. Approximately 13% of the administered dose is excreted via faeces, both unchanged and metabolized.

Half life

3-4 hours

Contra indication

Almotriptan should not be given to patients with ischemic heart disease or to patients who have symptoms or findings consistent with ischemic heart disease, coronary artery vasospasm, including Prinzmetal's variant angina or other significant underlying cardiovascular disease because

- It may increase blood pressure, it should not be given to patients with uncontrolled hypertension
- It should not be administered within 24 hours of treatment with another 5-HT1 agonist, or an ergotamine-containing or ergot-type medication like dihydroergotamine or methysergide.
- It is contraindicated in patients who are hypersensitive to almotriptan or any of its ingredients.

Adverse effects

Serious cardiac events, including myocardial infarction and coronary artery vasospasm, have occurred following the use of almotriptan malate. These events are extremely rare and most have been reported in patients with risk factors predictive of CAD. Events reported in association with drugs in this class have included coronary artery vasospasm, transient myocardial ischemia, myocardial infarction, ventricular tachycardia, and ventricular fibrillation.

Side effects

Almotriptan is generally well tolerated. The side effects are usually mild and do not last long. The following is not a complete list of side effects.

The most common side effects are

- Nausea
- Sleepiness
- Tingling or burning feeling (paraesthesia)
- Headache
- Dry mouth.

Drug interactions

- **Ergot-Containing Drugs**

These drugs have been reported to cause prolonged vasospastic reactions. Because there is a theoretical basis that these effects may be additive, use of ergotamine-containing or ergot-type medications (like dihydroergotamine or methysergide) and almotriptan within 24 hours of each other should be avoided.

- **Monoamine Oxidase Inhibitors**

  Co-administration of moclobemide resulted in a 27% decrease in almotriptan clearance and an increase in Cmax of approximately 6%.

- Other 5-$HT_{1B/1D}$ Agonists

  Concomitant use of other 5-$HT_{1B/1D}$ agonists within 24 hours of treatment with almotriptan is contraindicated.

- Selective Serotonin Reuptake Inhibitors/Serotonin Norepinephrine Reuptake Inhibitors and Serotonin Syndrome

Cases of life-threatening serotonin syndrome have been reported during combined use of selective serotonin reuptake inhibitors (SSRIs) or serotonin norepinephrine reuptake inhibitors (SNRIs) and triptans.

- Verapamil

Co-administration of almotriptan and verapamil resulted in a 24% increase in plasma concentrations of almotriptan.

POLYMER PROFILE

CHITOSAN (Pankaj Shard1*et.al, 2014)*

Non-proprietary names:

BP: Chitosan hydrochloride

PhEur: Chitosani hydrochloridum

Synonyms:

2-Amino-2-deoxy-(1,4)-β-D-glucopyranan; deacetylated chitin; deacetylchitin; β-1,4-poly-D-glucosamine; poly-D-glucosamine; poly-(1,4-β-D-glucopyranosamine).

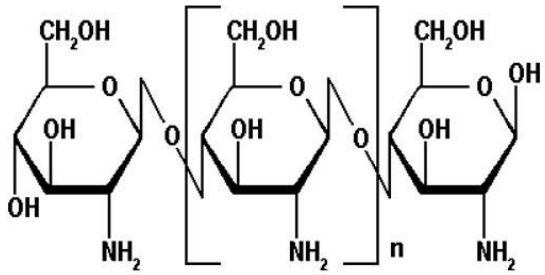
Chemical name and CAS registry number:

Poly-β-(1,4)-2-Amino-2-deoxy-D-glucose [9012-76=4]

Functional category:

- Coating agent
- disintegrant
- film-forming agent
- tablet binder
- Viscosity-increasing agent.

Structural formula



Applications in pharmaceutical formulation:

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated innumerous studies. These include controlled drug delivery applications, use as a component of Mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems, and use for gene delivery. Chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets, and coatings for liposomes. Furthermore, chitosan may be processed into drug delivery systems using several techniques including spray-drying, coacervation, direct compression, and conventional granulation processes.

Description:

Chitosan occurs as odourless, white or creamy-white powder or flakes. Fibre formation is quite common during precipitation and the chitosan may look 'cotton like'.

Acidity/alkalinity:

pH = 4.0–6.0 (1% w/v aqueous solution)

Density:

1.35–1.40 g/cm

Solubility:

It is sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric

and sulphuric acids). Upon dissolution, amine groups of the polymer become protonated, resulting in a positively charged polysaccharide and chitosan salts (chloride, glutamate, etc.) that are soluble in water; the solubility is affected by the degree of deacetylation. Solubility is also greatly influenced by the addition of salt to the solution. The higher the ionic strength, the lower the solubility as a result of a salting-out effect, which leads to the precipitation of chitosan in solution. When chitosan is in solution, the repulsions between the deacetylated units and their neighbouring glucosamine units cause it to exist in an extended conformation. Addition of an electrolyte reduces this effect and the molecule possesses a more random, coil-like conformation.

Typical properties

Chitosan is a cationic polyamine with a high charge density at pH <6.5; and so adheres to negatively charged surfaces and chelates metal ions. It is a linear polyelectrolyte with reactive hydroxyl and amino groups (available for chemical reaction and salt formation). The properties of chitosan relate to its polyelectrolyte and polymeric carbohydrate character. The presence of a number of amino groups allows chitosan to react chemically with anionic systems, which results in alteration of physicochemical characteristics of such combinations. The nitrogen in chitosan is mostly in the form of primary aliphatic amino groups. Chitosan therefore undergoes reactions typical of amines: for example, N acylation and Schiff reactions. Almost all functional properties of chitosan depend on the chain length, charge density, and charge distribution. Numerous studies have demonstrated that the salt form, molecular weight, and degree of deacetylation as well as pH at which the chitosan is used all influence how this polymer is utilized in pharmaceutical applications.

Stability and storage conditions

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place.

Incompatibilities

Chitosan is incompatible with strong oxidizing agents.

Safety

Chitosan is being investigated widely for use as an excipient in oral and other pharmaceutical formulations. It is also used in cosmetics. Chitosan is generally regarded as a nontoxic and non-irritant material. It is biocompatible with both healthy and infected skin. Chitosan has been shown to be biodegradable.

LD50 (mouse, oral) : >16 g/kg

Regulatory Status

Chitosan is registered as a food supplement in some countries.

SODIUM TRIPOLYPHOSPHATE

Non-proprietary names

BP: Sodium tripolyphosphate

Synonyms:

STP;s400;poly;STPP;armofos;polygon;tripoly;thermphos;thermphosn;rho diaphoslv

Chemical Name and CAS No.

Sodium tripolyphosphate (7758-29-4)

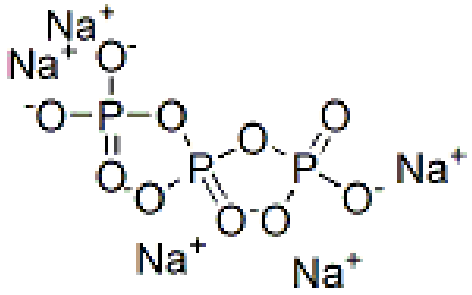
Molecular Formula:

Na5O10P3

Molecular Weight:

367.86

Molecular structure



Functional category

Modifier; emulsifier; buffer; chelating agent; stabilizer. Can also be used as soft water, pH regulator and thickening agent.

Description

Sodium tripolyphosphate is a colourless salt, which exists both in anhydrous form and as the hexahydrate.

Application in pharmaceutical formulation or technology

Sodium Tripolyphosphate is widely used as Dispersant in Pharmaceutical. It is used as a crosslinking agent.it is also used as a preservative and emulsifying agent.

MATERIALS AND EQUIPMENTS

Materials Used

Material	Source
Almotriptan Malate	Azakem Laboratories , Hyderabad
Chitosan	Sigma Aldrich, Mumbai
Sodium Tripoly Phosphate	Sigma Aldrich, Mumbai
Glacial Acetic Acid	Sigma Aldrich, Mumbai

Equipments Used

Equipments	Model/ Company
pH Tester	Eutech Instruments
Magnetic Stirrer	REMI – 2MLH
Laboratory Centrifuge	REMI
Transmission Electron Microscopy	CUSAT, Cochin
UV – Visible Spectrophotometer	Jasco v 530
Dissolution Apparatus	Electro lb TDT 08L
FT-IR Spectrophotometer	Jasco FTIR 4100
Differential Scanning Calorimeter	CUSAT, Cochin
Thermoanalyzer	CUSAT, Cochin
Diffractometer	CUSAT, Cochin

EXPERIMENTAL METHODS

I. PREFORMULATION STUDIES

Solubility studies

Weighed accurately 10mg of the drug. Dissolved it in 5ml of the following solutions

i. Water
ii. Glacial acetic acid
iii. Phosphate buffer

Preparation of Calibration curve of almotriptan malate

Preparation of pH 6.5 phosphate buffer (IP 2007 vol 1)

Dissolved 60.5g of disodium hydrogen phosphate and 46g of potassium dihydrogen phosphate in water, add 100ml of 0.02m disodium edetate was added and 20 mg of mercuric Chloride was diluted with water to produce 1000ml.

Calibration curve of almotriptan malate

100mg of almotriptan malate was accurately weighed into 100ml volumetric flask and dissolved in methanol. The volume was made upto ml with 6.5 phosphate buffer solution to get a concentration of 1000µg/ml from this, 10ml was withdrawn and diluted to 100ml with 6.5Ph phosphate buffer solution to get a concentrstion100µg/ml. From the standard stock solution 0.2ml, 0.4ml, 0.8ml and 1ml were withdrawn and volume was made upto pH 6.5 phosphate buffer to give a concentration of 2,4,6,8, and 10 µg/ml. Absorbance of these solutions were measured against blank of pH 6.5 phosphate buffer solution at 227 nm.

FTIR studies (Sagar S.Jadhav *et al*., 2013)

The FT-IR studies are conducted by potassium bromide disc pellet method. 10mg of the samples and 400mg of KBr were taken in a mortar and triturated. A small amount of the triturated sample was taken into the pellet maker. It was compressed at 10kgm/cm^2 using a hydraulic press. The pellet was taken on the sample holder. It was then scanned from 4000cm^{-1} to 400cm^{-1} in FT IR spectrophotometer. Samples were prepared for drug and excipients also. The spectra obtained were compared and interrupted for functional group peaks

II. PREPARATION OF NANOPARTICLES (Saugat Adhikari *et. al*.,2012)

Chitosan biodegradable nanoparticles were prepared based on the ionic gelation of chitosan with sodium tripolyphosphate anions. Chitosan was dissolved in aqueous acetic solution at concentration (1%w/v). The concentration of acetic acid in aqueous solution was 1.5 times that of chitosan. Blank nanoparticles are obtained upon the addition of 4ml sodium tripolyphosphate aqueous solution with concentration of 0.5, 1.0, 1.5 mg/ml under magnetic stirrer at room temperature. Drug loaded nanoparticles were formed upon incorporation of 4ml sodium tripolyphosphate into 10ml chitosan solution with known concentration containing almotriptan. The nanoparticle suspension obtained was centrifuged at 10000 rpm for 30min, washed and dried

Table 1 Formulation Of Chitosan Nanoparticle

p	Batch code	Chitosan (mg/ml)	Sodium Tripolyphosphate (mg/ml)	Almotriptan Drug (mg)
1	B_0	1	0.5	0
2	B_1	1	0.5	2
3	B_2	2	1	2
4	B_3	3	1.5	2

III. STATISTICAL OPTIMIZATION USING 2^3 FACTORIAL DESIGN (Girotra PritiHansraj *et al*,. 2013)

Preliminary experiments, performed to determine the major factors affecting the particle size and entrapment efficiency of the nanoparticles, suggested that the concentration of polymer chitosan, cross linking agent and surfactant tween 80, were the most significant factors. A 2^3 factorial design was selected to evaluate the effect of these three factors each at two levels, on the dependent variables viz. particle size and entrapment efficiency. A total of four experimental runs for the estimation of pure error were developed and the NPs were formulated. Details of the experimental design have been summarized in Table. The experimental design and all the regressions were performed using Minitab 17.

IV. CHARACTERIZATION OF NANOPARTICLE

Morphological characterization and particle size determination of nanoparticles (Sagar S.Jadhav *et al.,* 2013*)*

Particle morphology and particle size were examined by transmission electron microscopy (TEM). The samples were immobilized in the carbon/ formvar coated copper grids. They were dried at room temperature, and then were examined using a TEM without being stained.

Determination of almotriptan loading capacity and entrapment efficiency (Sagar S.Jadhav *et al.,* 2013*)*

The entrapment efficiency can be determined by measuring the concentration of the free drug in the dispersion. Entrapment efficiency and drug loading capacity of nanoparticles in different formulation are determined by ultra-centrifugation of samples at 10,000 rpm for 30 min. The amount of free almotriptan was determined in clear supernatant by UV spectrophotometry at 227nm using supernatant of non-loaded (blank) nanoparticles as basic correction. The drug loading capacity of nanoparticles and entrapment efficiency (EE) of the process were calculated from equations 1 and 2 indicated below:

$$Entrapment\ Efficiency(EE\%) = \frac{total\ amount\ of\ drug - free\ drug}{total\ drug} \times 100$$

$$Drug\ loading\ capacity = \frac{total\ amount\ of\ drug - free\ drug}{total\ weight\ of\ nanoparticles} \times 100$$

***Invitro* drug release study of the prepared formulations** (Sagar S.Jadhav *et al.,* 2013*)*

The *in vitro* drug release study of the optimized batch is to be performed in a USP Type II (Paddle type) apparatus using the principle of diffusion through the dialysis membrane. Drug loaded nanoparticles redispersed in 2 ml phosphate buffer (pH 6.5), were placed in a dialysis bag soaked in distilled water 1Hour prior to its use, and the bag was tied to the paddle of the apparatus. The paddle was rotated at 100 rpm and the dissolution media was 500 ml phosphate buffer pH 6.5 maintained at 37 ˚C±1+C. Sample aliquots of 5 ml were collected at specific time intervals and were replenished with equal amount of fresh dissolution medium. The samples were analysed, spectrophotometrically at λmax = 227 nm against suitable blank and amount of drug released at various time intervals were calculated. The results were expressed in terms of average % drug release± standard deviation.

***In vitro* drug release kinetics**

The drug release kinetics of almotriptan nanoparticles was determined by plotting the following kinetic models, using the data collected from in-vitro release studies. (zero order, first order and Higuchi equations). The mechanism of drug release was determined by using Korsmeyer-Peppas equations.

a) ***Zero-Order Kinetics***:

Cumulative amount of drug released was plotted against time.

$C = K_{0T}$

where K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration Vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axis. This kinetics describes concentration independent drug release from the formulations.

b) First order kinetics:

First order as log cumulative percentage of drug remaining vs time. This kinetics describes concentration dependent drug release from the formulations.

$$\text{Log } C = \text{Log } Co - K_t / 2.303$$

where C0 is the initial concentration of drug, k is the first order constant, and t is the time.

c) Higuchi's Model:

Higuchi's model as cumulative percentage of drug released vs. square root of time.

$$Q = K_{t1/2}$$

where K is the constant reflecting the design variables of the system and t is the time in hours. This model describes the release of drug on the basis of Fickian diffusion as a square root of time dependent process from swellable matrix.

d) Korsmeyer-Peppas Equations:

The mechanism of drug release, the first 60% of drug release were plotted in Korsmeyer equation log cumulative percentage of drug released vs log time, and the exponent n was calculated through the slope of the straight line,

$$M_t / M_\infty = Ktn$$

Where M_t/M_∞ is the fractional solute release, t is the release time, K is a kinetic constant. Characteristic of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers. For cylindrical matrix tablets, if the exponent n = 0.45, then the drug release mechanism is Fickian diffusion, and if $0.45 < n < 0.89$, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release (Balaiah. A et al.,2012).

Differential Scanning Calorimetry (DSC) (Sagar S.Jadhav *et al*., 2013)

DSC thermograms of pure drug almotriptan malate, drug loaded optimized formulation and optimized formulation without drug were recorded on a DSC equipment to determine the physical nature of the drug and the polymers/carriers in the formulation. 4–5 mg of each of the samples were weighed in aluminium pans and then crimped. The samples were scanned in the temperature range of 25–400 ◦C at a heat flow rate of 10 ◦C/min. The sample cell was continuously purged with nitrogen at a flow rate of 100 ml/min. The data is then analysed.

Thermogravimetric analysis (TGA) (Sagar S.Jadhav *et al*., 2013)

Thermoanalyzer was used for acquiring the thermo grams of drug almotriptan malate, Dummy nanoparticle and optimized formulation. The equipment was operated under nitrogen atmosphere, from 30◦ to 500 ◦C at a heating rate of 10 ◦C/min. The results were expressed in terms of per cent weight loss with respect to temperature.

Powder X-ray diffraction study (Sagar S.Jadhav *et al*., 2013)

The XRD patterns of pure drug, dummy nanoparticle and optimized nanoparticle are to be measured on a diffractometer. Monochromatic radiation (λ = 1.5418A˚), at 40 kV and 40 mA, were used as the X-ray source. The diffractograms were recorded between 20º and 70º with an increasing step of 0.02º and 2 s as the time for each step.

Mucoadhesive Test (Sagar S.Jadhav *et al*., 2013)

A piece of goat nasal mucosa was cleaned and placed in Krebs solution in Petri plate. It was washed with distilled water, and then accurately weighed RZB loaded CS Nanoparticles equivalent to 100 mg were spread on to it. The mucosa was kept aside for 5 min. Then surface was washed with phosphate buffer pH 6.5.

Nanoparticles retained on mucosal surface after first washing were removed with rinsing thoroughly by phosphate buffer 6.5. The solution was stirred to dissolve drug, filtered and absorbance was recorded. The amount of adhered microspheres was estimated as the difference between the amount of applied microspheres and the amount of flowed microspheres. The percent mucoadhesion was calculated using the following

$$percentage\ of\ mucoadhsesion = \frac{amount\ of\ drug\ in\ wash\ out\ liquid}{actual\ amount\ of\ drug\ in\ nanoparticles} \times 100$$

V. STABILITY STUDIES

A study was also carried out to assess the stability of almotriptan malate loaded chitosan nanoparticles. For this purpose, samples were kept in borosilicate glass vials and stored at room temperature, in a refrigerator (5 ± 1°C), and 45 ± 1°C (75% relative humidity) in the stability chamber. Samples were analysed at the intervals of 0, 7, 14, 21, 28, 35, and 45 days for their changes in physical appearance and for the drug content.

RESULTS AND DISCUSSION

I. PREFORMULATION STUDIES

Solubility studies

Solubility studies was carried out to select a suitable solvent to dissolve the drug and to select the dissolution medium.

Table 2: Solubility Studies

Sl. No	Solvent	Solubility
1	Water	Soluble
2	Phosphate buffer pH 6.5	Soluble
3	Glacial acetic acid	Soluble

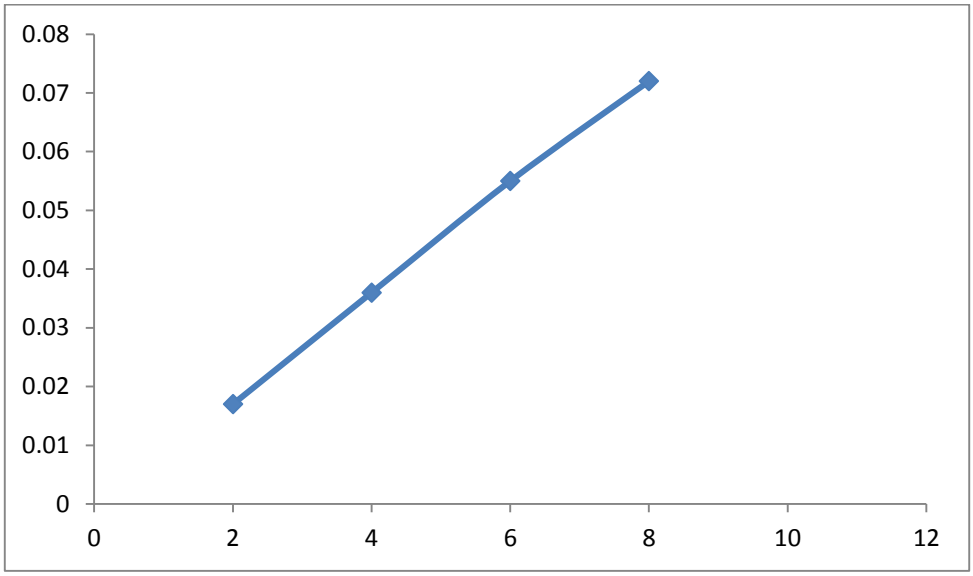
Calibration curve of almotriptan malate

In the validation studies, it was found that the estimation of almotriptan malate by spectrophotometric method at 227 has good reproducibility, at the concentration between 2-10µg/ml. correlation between concentration and absorbance was found to be 0.9997845 which is closer to 1.

Table 3: Calibration curve of almotriptan

Si No	Concentration	Absorbance At 227nm
1	2	0.017
2	4	0.036
3	6	0.055
4	8	0.072
5	10	0.089

Graph 1: Calibration curve of almotriptan malate



FTIR studies

The compactability studies between the drug and polymers were evaluated by using IR matching approach.

Graph 2: FTIR Of Almotriptan

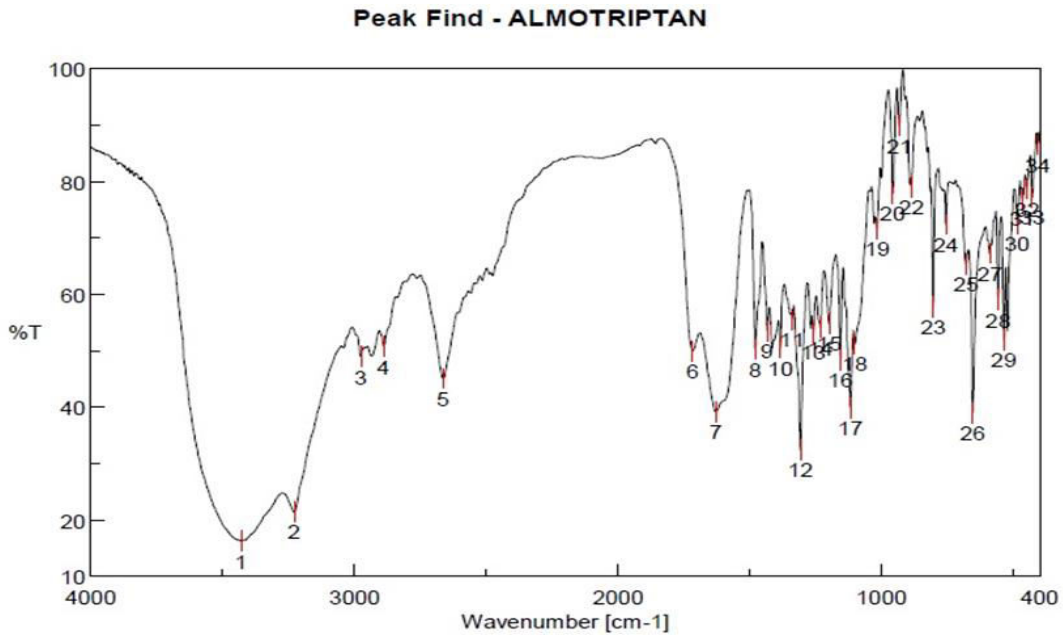


Table 4: FTIR Interpterion Of Drug Almotriptan Malate

FTIR INTERPETION OF DRUG ALMOTRPTAN MALATE					
Materials	**Function Group**		**Type of Vibration**	**Characteristic Absorptions (Cm-1)**	**Test Absorption**
Almotriptan malate	CH		stretching	2840-3000	2976.59
			bending	1350-1480	1433.82
	NH		stretching	3400-3500	3429.78
	C=C			1400-1600	1433.82
	S=O			1030-1060	1038.45
	COOH	0H		2500-3300	2563.21
		C-O		1210-1320	1229.12

Graph 3: FTIR of almotriptan and chitosan

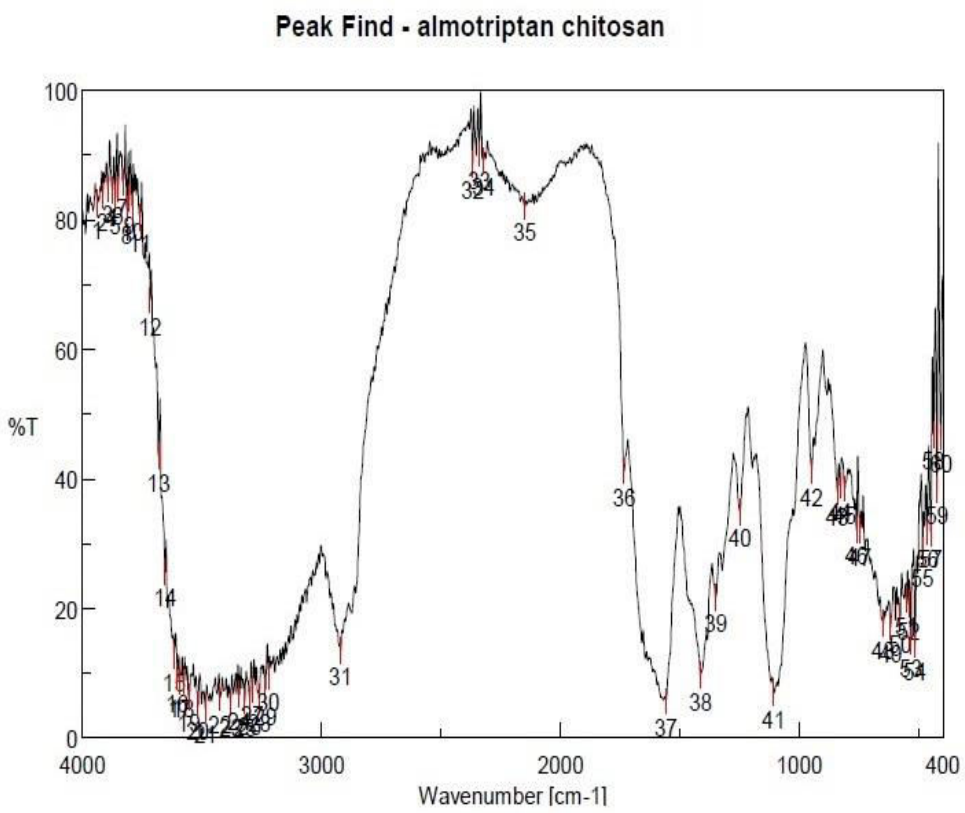


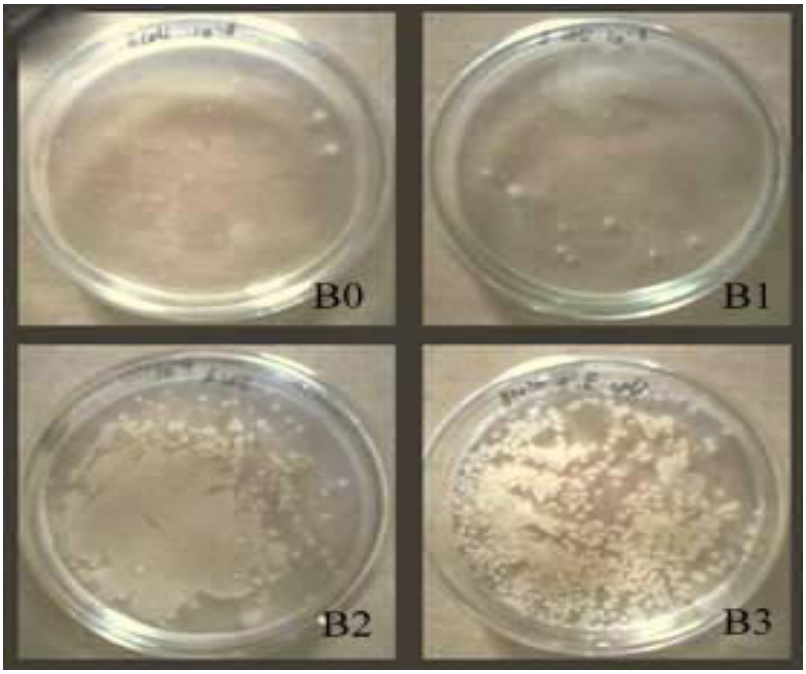
Table 5: FTIR interpretation of almotriptan and chitosan

FTIR INTERPETION OF DRUG ALMOTRPTAN MALATE AND CHITOSAN				
Materials	**Function Group**	**Type of Vibration**	**Characteristic Absorptions (Cm-1)**	**Test Absorption**
Chitosan	OH	Stretching	3200-3520	3336
	C-O-C	Stretching	1850 -2150	1187.58
	NH_2	Stretching	3300- 3500	3455
	CH	Stretching	2850-2950	2943

II. PREPARATION OF ALMOTRIPTAN NANOPARTICLE

3 formulation and a blank formulation of almotriptan malate loaded chitosan nanoparticles were prepared using ion gelation method by using sodium tripolyphosphate as cross linking agent.

Figure 6 : Prepared Nanoparticles



III. STATISTICAL OPTIMIZATION USING 2^3 FACTORIAL DESIGN

Eight formulations were prepared in accordance with the design layout generated by 2^3 full factorial design. The influence of three formulation parameters viz. concentration of chitosan, concentration of STPP and amount of tween80 on the five response variables, viz. particle size and entrapment efficiency was investigated. The results of the responses have been presented in below figures. The mean particle size of all the formulation batches ranged between 100 - 234. The entrapment efficiency of the formulations varied between 32% and 81%.

The equations obtained by the regression analysis for the two response variables are as follows:

$$partcle\ size = 190.6 + 19.9 chitosan\ concentration - 15.6 STPP\ concentration + 20.1\ tween80\ concentration$$

$$entrapment\ efficiency = 58.75 - 6.75 chitosan\ concentration + 7.25\ STPP concentration - 6.50\ tween\ 80\ concentration$$

The cube plots, highlighting the effect of three formulation factors on the two response variables, have been depicted in figure. The effect of chitosan concentration, STPP concentration and tween 80 concentration on the particle size of the formulation has been represented in Fig.7. It was observed that particle size increases with the increase in chitosan. The effect of chitosan concentration, STPP concentration and tween 80 concentration on the particle size of the formulation has been represented in Fig.8. Presence of chitosan was more prominent factor affecting entrapment efficiency which may be ascribed to rigidization of nanoparticle.

Figure 7: Cube Plot For Particle Size

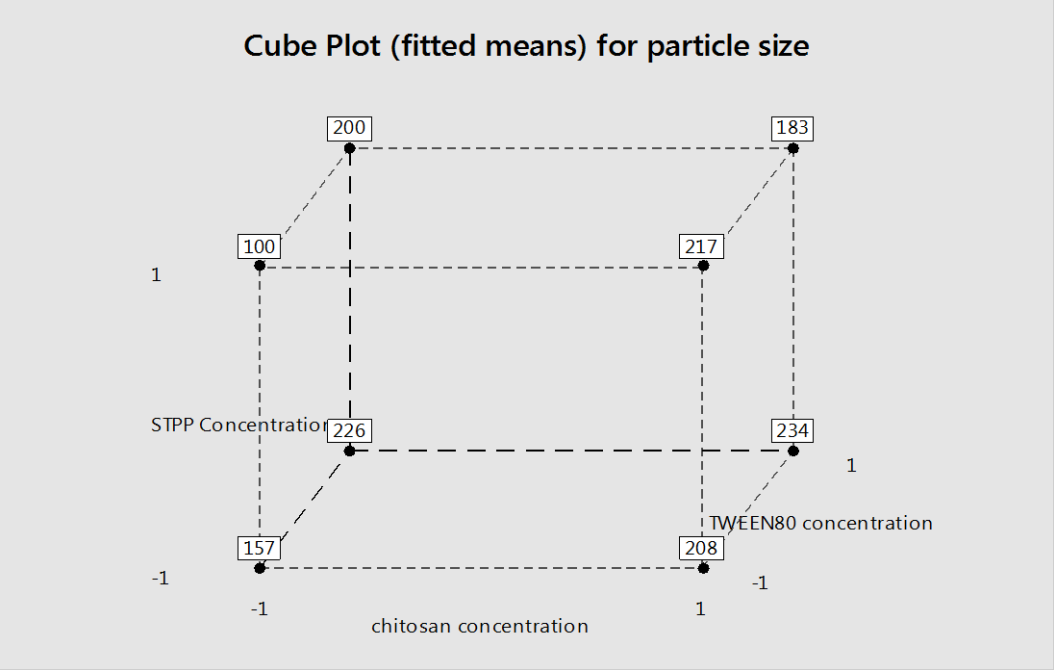
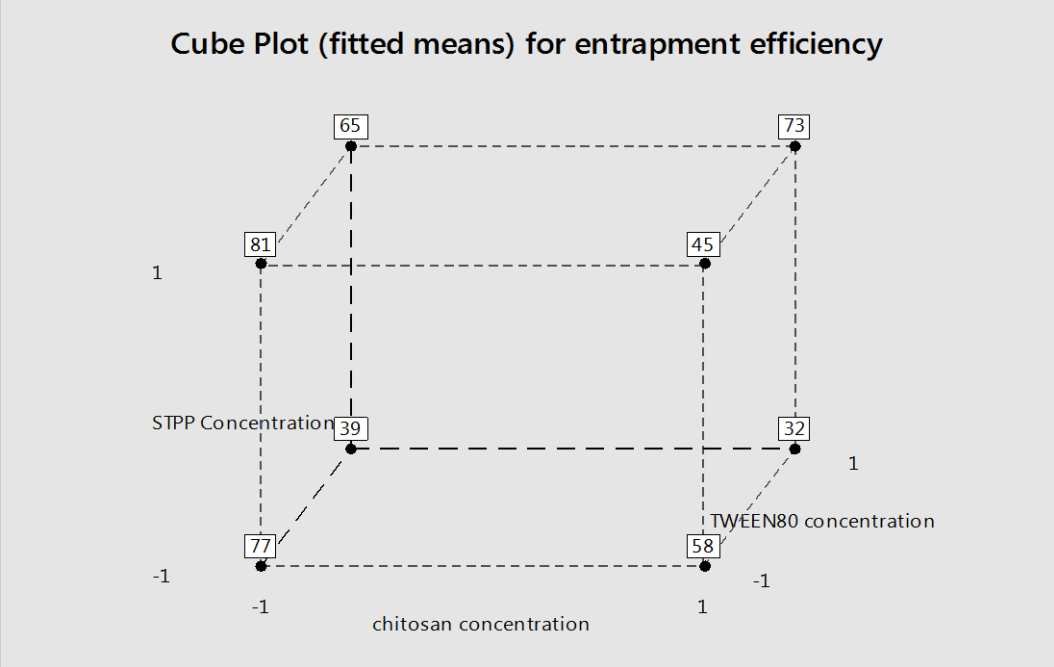


Figure 8: Cube Plot For entrapment efficiency



IV.CHARACTERIZATION OF NANOPARTICLES

1. Morphological characterization and particle size determination of nanoparticles

Particle morphology and particle size were examined by transmission electron microscopy (TEM).

The TEM image of drug loaded optimized chitosan nanoparticle, displayed below, reveals that the particles were

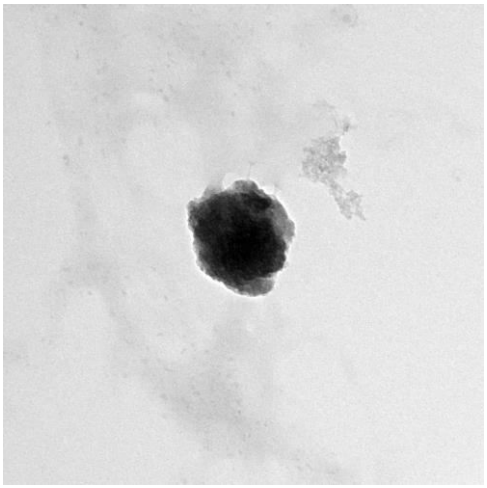
- Segregated
- Uniform in size
- Spherical in shape.

The nanoparticles were within a narrow size distribution range in B_0, B_1 and B_2 batches and particle size slightly increased in B_3 batch with high concentration of each composition.

Table 6: Particle size range

Sl. No	Batch code	Size range (nm)
1	B_0	100nm
2	B_1	100nm
3	B_2	157nm
4	B_3	183nm

Figure 9: TEM Image Of B_0

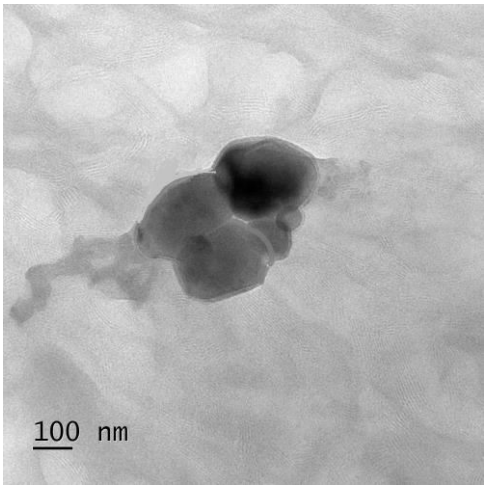


B_0

SHAPE: SPHERICAL

SIZE: 100NM

Figure 10: TEM Image Of B_2

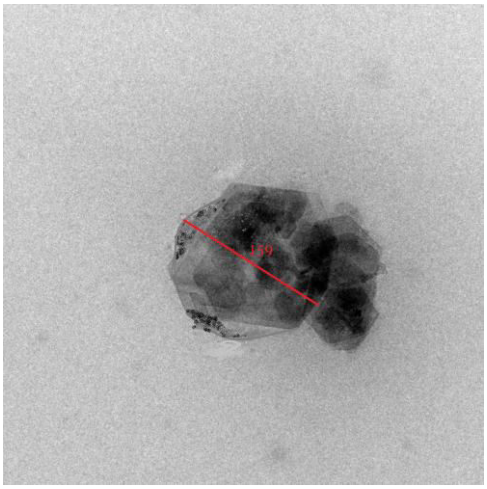


B1

SHAPE – SPHERICAL

SIZE -100nm

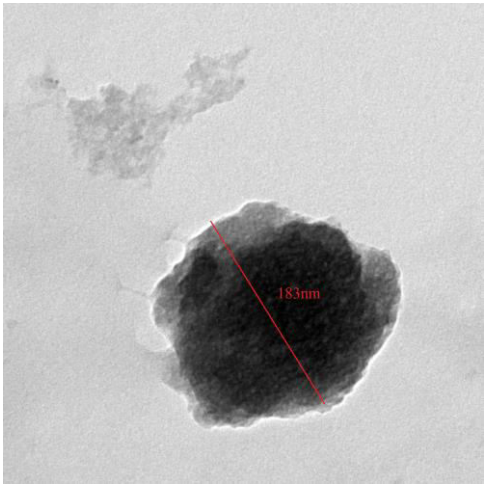
Figure 11: TEM Image of B_2



B_2 SHAPE: SPHERICAL

SIZE 159 NM

Figure 12: TEM Image Of B_3



B_3

SHAPE: SPHERICAL

SIZE :183NM

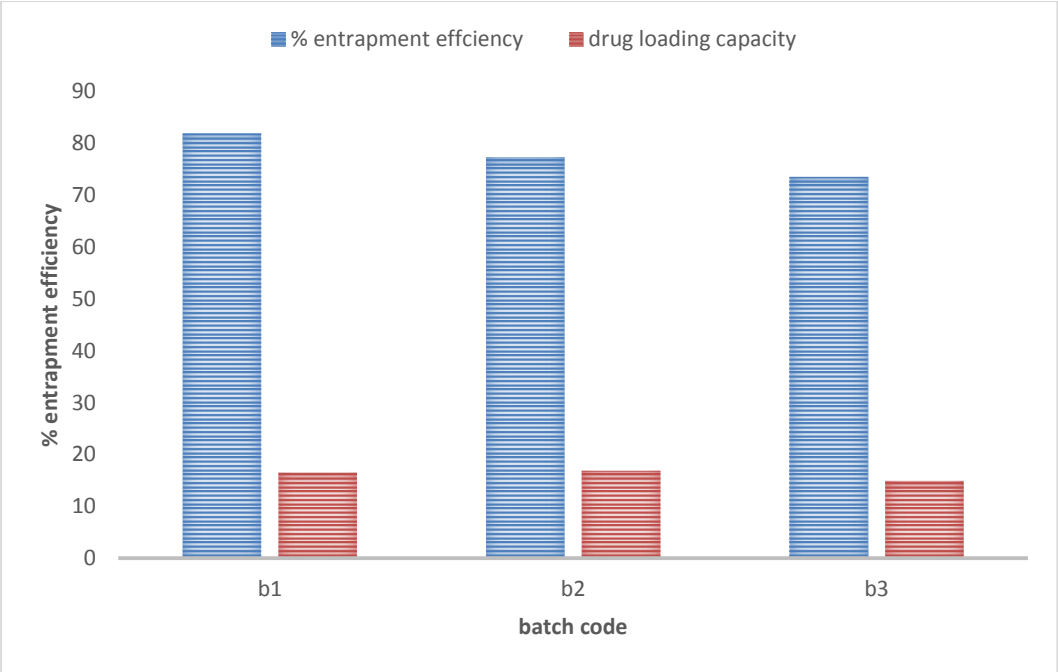
Determination of almotriptan loading capacity and entrapment efficiency

Entrapment efficiency means that the amount of drug in the precipitate did not change via different formulations. But, for constant amount of entrapped drug, upon increasing surfactant concentration the number of excipients increases, which results in reduced drug loading.

Table 7: Entrapment Efficiency And Drug Loading Capacity

Si no	Batch code	Entrapment efficiency (EE%)	Almotriptan Drug Loading Capacity
1	B_1	81.9%	16.38
2	B_2	77.35%	15.47
3	B_3	73.5%	14.79

Graph 4: Entrapment Efficiency And Drug Loading Capacity



Percentage Entrapment efficiency was found to be highest for B_1 formulation which is 81.9% whereas the lowest entrapment of drug was found to be for B_3 having 73.5%.

Drug loading capacity was more for B_1 and less for B_3.

***Invitro* drug release study of the prepared formulations**

The *in-vitro* release profile of ZMT from different formulations is shown in figure the percent drug release was not same for all the formulations.it varies with time.

The release profile for chitosan biodegradable nanoparticles was obtained in phosphate buffer solution, pH 6.5. Initially the chitosan nanoparticles showed immediate release of almotriptan malate, with about 60% ZMT being released in 45 minutes which may be due to release of drug from the surface nanoparticles, followed by slow release of drug which is encapsulated in CBN. When the particle size of drug loaded chitosan, nanoparticles is smaller, surface area tends to become larger and hence the drug release is faster.

Table 8: Cumulative percentage release studies

Time (min)	Cumulative percentage release		
	Batch 1	Batch 2	Batch 3
0	0	0	0
5	37	32	28
10	45	43	39
15	54	51	48
30	57	53	50
45	66	60	58
60	71	65	61
75	75	71	66
90	79	75	72

Graph 5: *In vitro* drug release studies

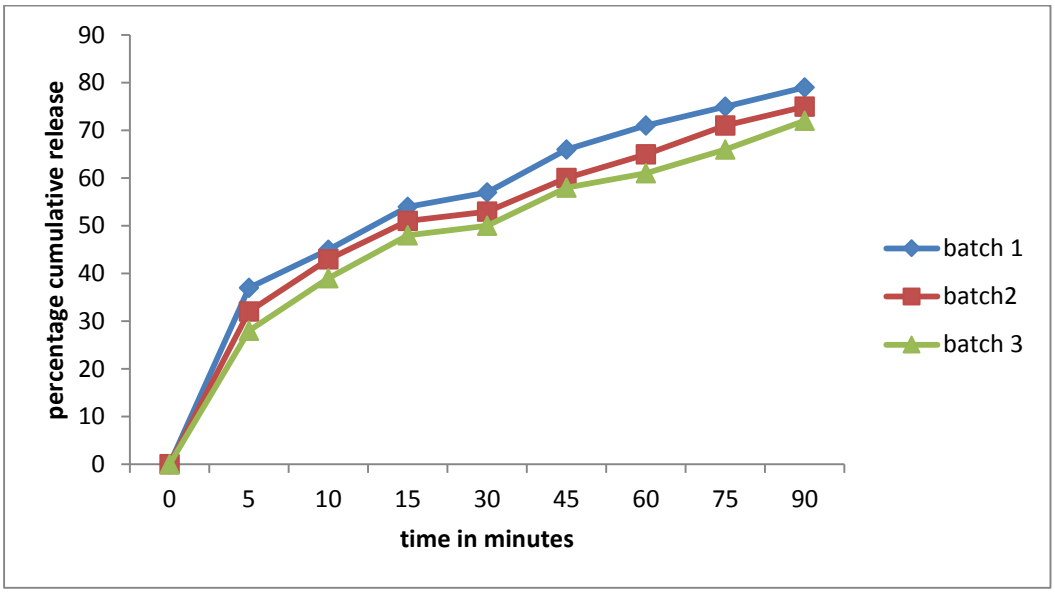


Figure 13: Dialysis membrane 150 with nanoparticles

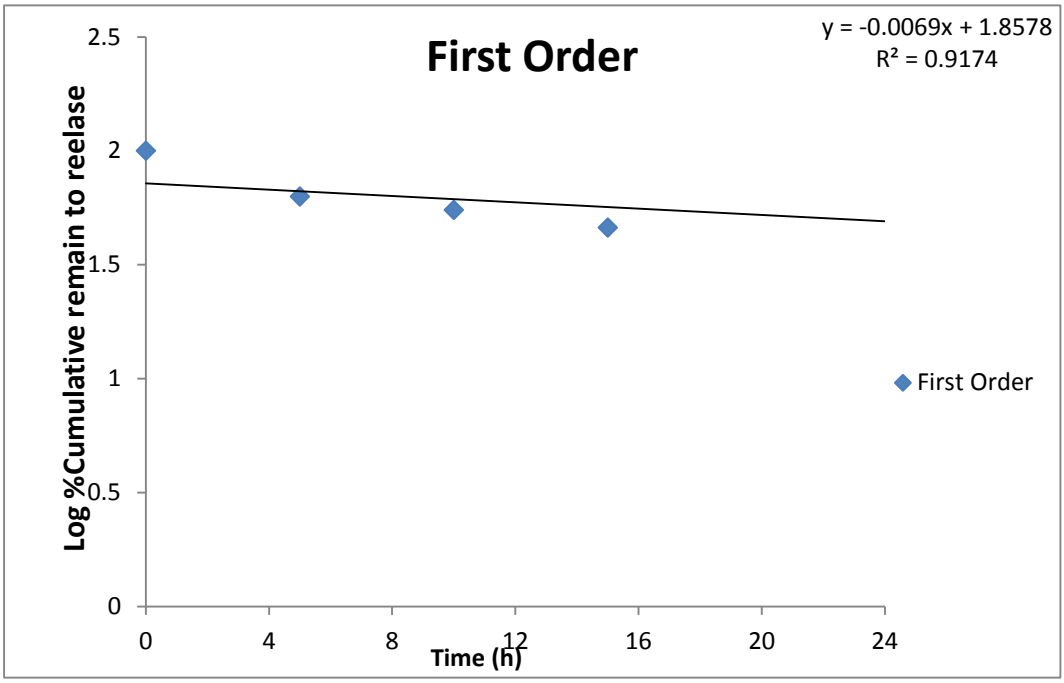
***Invitro* drug release kinetics of the optimized formulations**

To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained from *in-vitro* drug release from optimised formulation were fitted to model's representation zero order, first order, Higuchi and Korsemeyer-Peppas. The data indicates a lesser amount of linearity when plotted by the zero-order equation. Hence, it can be concluded that the major mechanism of drug release follows first order kinetics. Understanding the mechanism of drug release is possible by configuring the data into mathematical modelling of Korsmeyer-Peppas plots. Plotting of graph using percentage release vs. square root of time, revealed linearity. Data based on the first order models usually provide evidence to the diffusion mechanism of drug release from sustained release delivery systems.

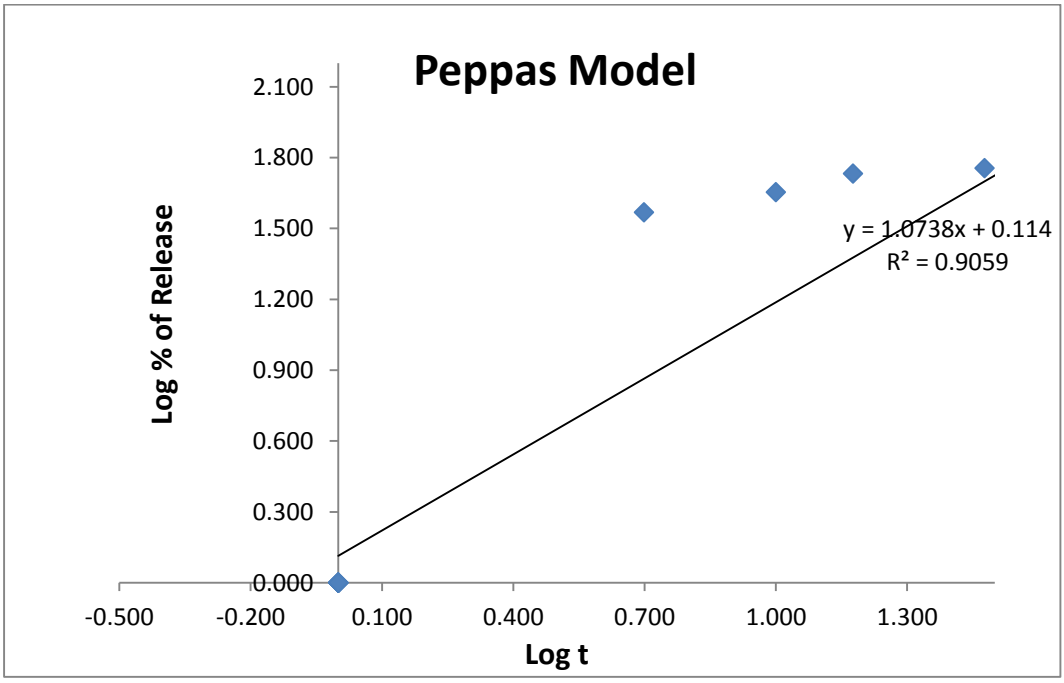
Kinetic studies of batch 1 (B_1)

Graph 6: Zero order kinetics of B_1

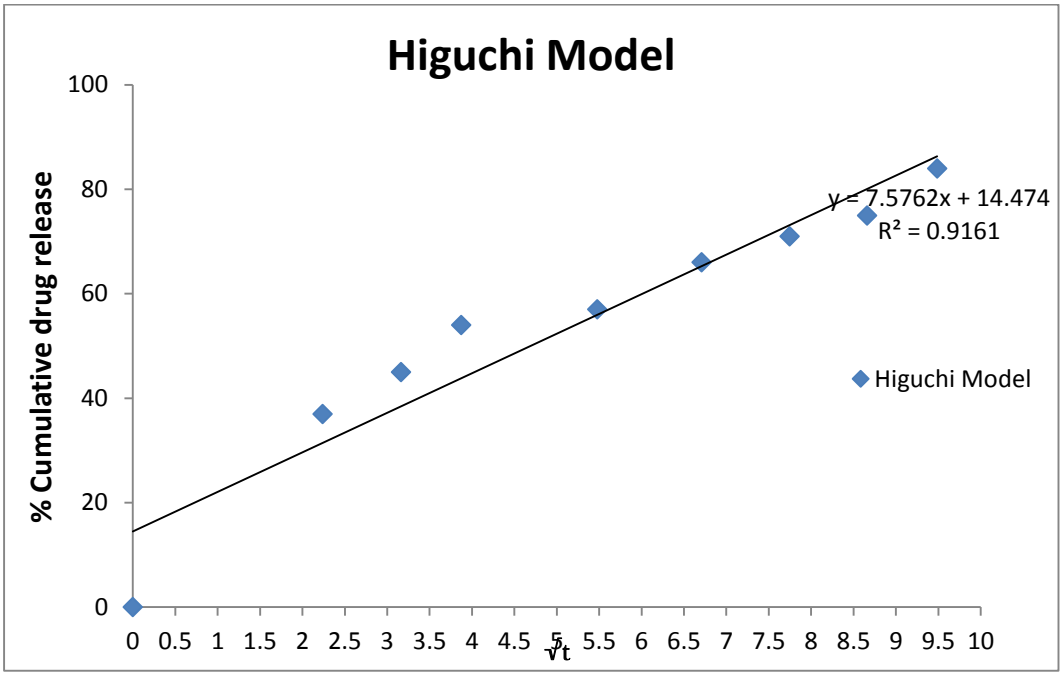
Graph 7: First order kinetics of batch 1



Graph 8: peppas model of batch 1



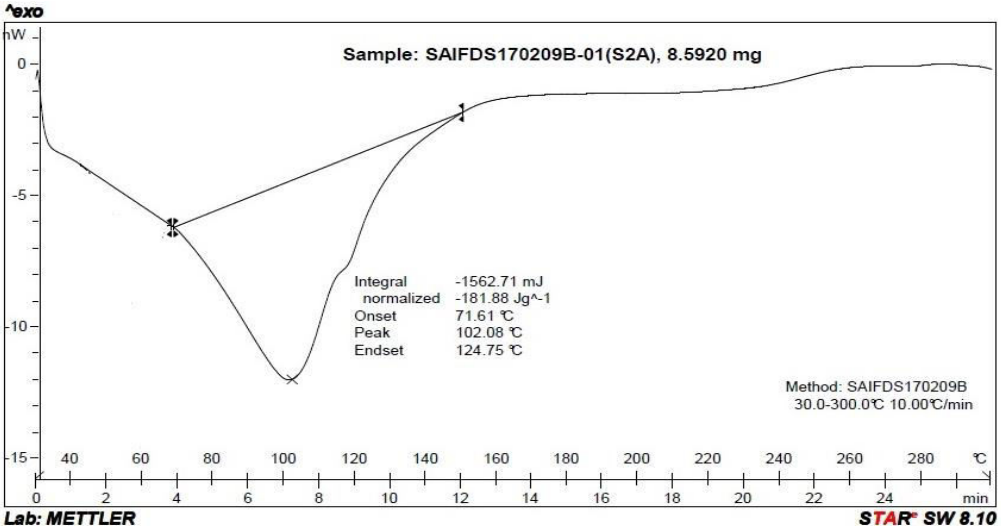
Graph 10: Higuchi model of batch 1



2. Differential scanning calorimetry

The DSC thermograms reveal the melting point and the crystalline or amorphous behaviour of the drug and the carriers.

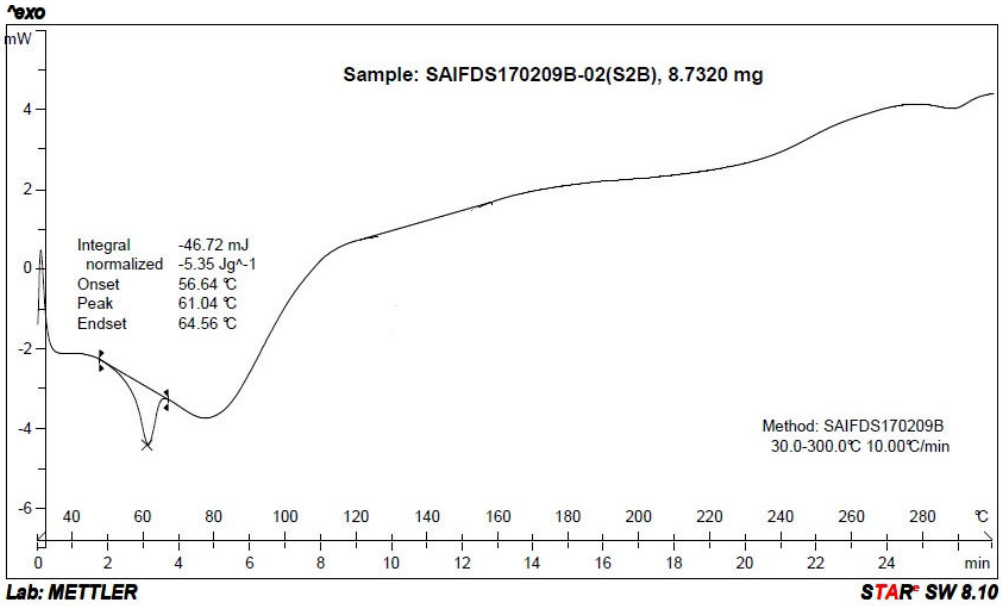
Graph 11: DSC Thermogram Of Drug Almotriptan



Thermal analysis indicated that the DSC scan of the drug presented a sharp endothermic peak. A sharp endothermic peak was obtained at 124.75°C in the thermogram of the pure drug almotriptan malate, indicating

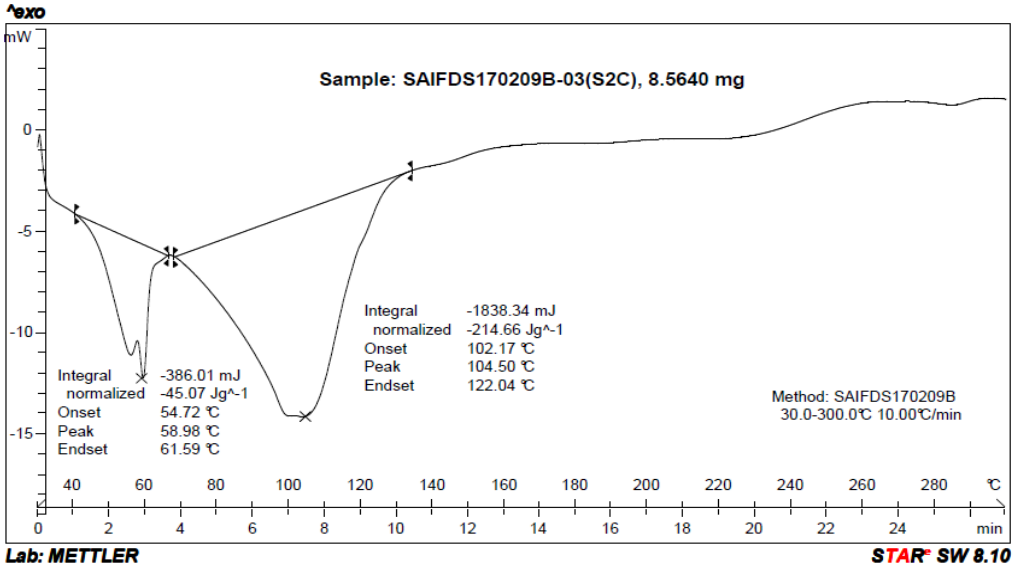
- its melting point temperature
- crystalline state.

Graph 12: DSC Thermogram Of Almotriptan Loaded Nanoparticles



- The endothermic peaks at 64ºC in the thermogram of blank nanoparticles corresponded to the melting point of sodium tripolyphosphate, present in the formulation.
- The melting point peaks of chitosan were not observed because of their amorphous nature.

Graph 13: DSC Thermogram Of Blank Nanoparticles



DSC spectrum of Chitosan Nanoparticles loaded with drug almotriptan does not exhibit the sharp peak of almotriptan malate. Thus, we can have concluded that in the Chitosan nanoparticles of almotriptan malate, drug was in partial crystalline state in chitosan nanoparticles and there is no interaction between drug and polymer.

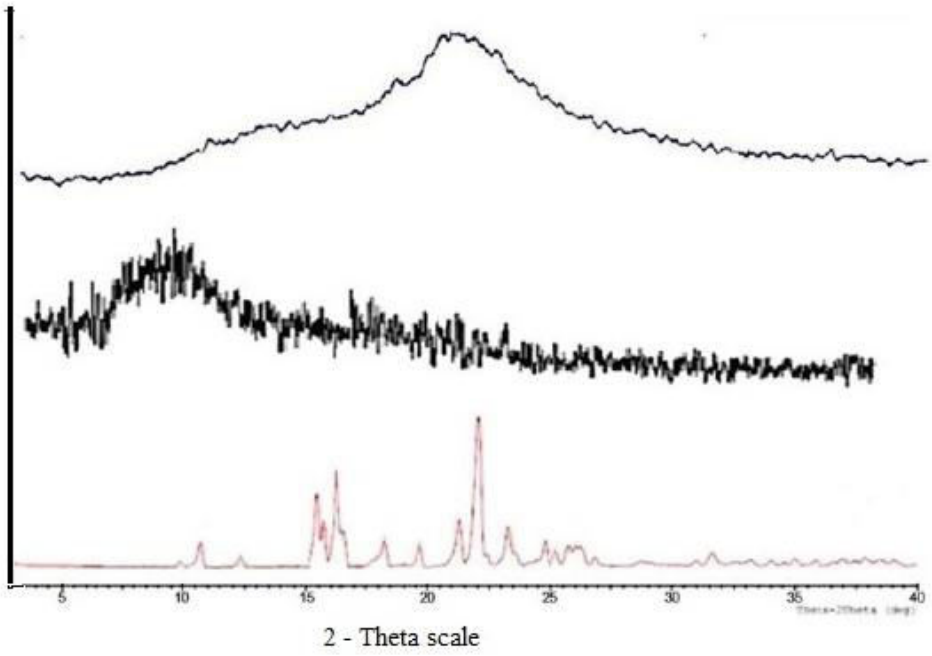
Thermogravimetric analysis

Result awaiting

Powder X-ray diffraction study

XRD studies are useful to investigate the crystallinity of drug in the chitosan nanoparticles. The X-ray diffractograms recorded for pure Almotriptan malate, blank nanoparticles and drug-loaded nanoparticles are presented. ALM revealed characteristic intense peaks at 2θ of 16°, 17° and 22° which are due to crystalline nature of ALM. However, in case of blank nanoparticles and drug-loaded chitosan nanoparticles no intense peaks were observed between 2θ of 16°, 17° and 22° indicating amorphous nature of the drug substance after entrapment into chitosan nanoparticles. It can be concluded that, drug particles are dispersed at the molecular level in the polymer matrices since no indication about the crystalline nature of the drug was observed in the drug-loaded chitosan nanoparticles.

Graph 14: Powder Diffraction Studies Of Drug, Drug Loaded NP, Blank NP



Mucoadhesive Test

Mucoadhesion studies were performed to ensure the adhesion of formulation to the mucosa for a prolonged period at the site of absorption. The results of the mucoadhesion studies are shown in the below table. The results indicated that amount of chitosan and volume of sodium tripolyphosphate was directly proportional to mucoadhesion strength. This could be attributed to the availability of a high amount of polymer for interaction with mucus.

Table 9: Percentage Mucoadhesion Of The Drug Loaded NP

Sl. No	Batch code	Percentage mucoadhesion (%) Of the drug loaded NP
1	B_1	69
2	B_2	61
3	B_3	54

Graph 14: Percentage Mucoadhesion Of The Drug Loaded NP

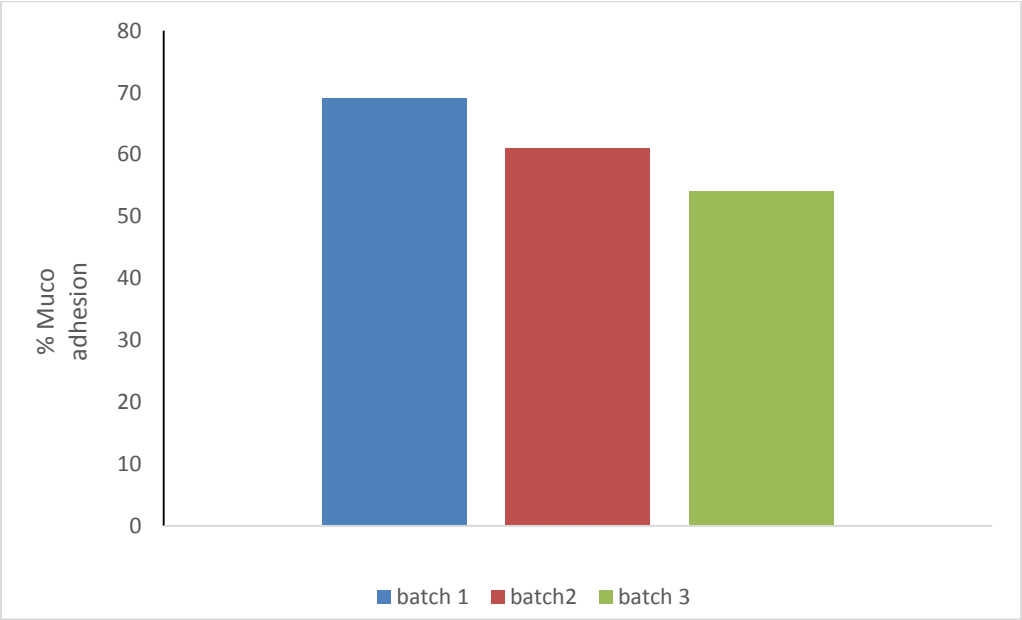


Fig 14: Nasal mucosa in kreb's solution

Fig 15: Nasal mucosa in phosphate buffer pH 6.5

Fig 16: Nasal mucosa with drug loaded chitosan nanoparticles

V. STABILITY STUDIES

The nanoparticles were taken and observed their physical properties at an interval 0f 7, 14,21,28,35 and 45 days. no change was observed in their physical appearance. Colour remains the same. The drug content remains unchanged in 5 ± 1°C and room temperature.

Table 11: Drug Content in Stability Studies

Si.no	Sampling interval days	Drug content		
		5 ± 1°C	**Room temperature**	**45 ± 2°C**
1	7	100	100	100
2	14	99.5	99.5	99.5
3	21	99.2	98.4	98.3
4	28	99.1	97.4	86.5
5	35	98.8	96.5	77.9
6	45	98.1	94.3	74.3

SUMMARY AND CONCLUSION

Nanoparticles are designed to improve the pharmacological and therapeutic properties of conventional drugs. Due to small dimensions, nanoparticles can cross the blood-brain barrier (BBB) and operate on cellular level. There is a wide range of nanoparticulate materials and structures being developed for the delivery of therapeutic compounds. Each has its own advantages, but as these nanoparticles become optimized for their specific application, the outcome will be better-controlled therapy because of targeted delivery of smaller amounts of effective drugs to the required sites in the body. This is being made possible using advanced material, improved control of particle size, and better understanding of interface between the biological and material surfaces, and their effects in vivo.

Almotriptan malate is an antimigraine drug. It exerts its pharmacological action by vasoconstriction of the meningeal arteries, inhibition of release of the neurotransmitters from neurons in brain, prevention of the nociceptive transmission and reduction of the activity of trigeminal nerves.

So, the present work was aimed to develop a novel approach for nose to brain targeted chitosan nanoparticle loaded with almotriptan malate for the treatment of migraine.

Chitosan biodegradable nanoparticles (CBN) of almotriptan malate were prepared by the ionic gelation method using polyanion sodium tripolyphosphate (STPP) as a cross-linking agent and their in-vitro characteristics were studied. The different formulations with varying concentration of chitosan and STPP was prepared. Solid state analysis was undertaken using thermal methods (DSC/MDSC), and Fourier transform infra-red spectroscopy (FT-IR) for compatibility studies. The particle size and morphology was determined by transmission electron microscopy. FTIR showed no significant interactions between chitosan and drug, STPP and drug.

The present study demonstrated the optimization of almotriptan malate loaded chitosan nanoparticles, using 2^3 full factorial design so as to develop an effective strategy for the decreased particle size, increased entrapment efficiency with maximum brain targeting efficiency. Optimization aided in understanding the interaction of formulation parameters, which can be exemplified by increased particle size with decreased chitosan concentration. Chitosan led to the increase in entrapment efficiency and STPP as well as Tween 80, conjointly, assisted in drug deposition in brain, probably by surpassing the blood brain barrier owing to their lipophilic and surfactant properties

Particle size distribution analysis confirmed the size ranges, with a narrow size distribution. TEM indicated smooth and spherical Nanoparticles.

The drug was chemically stable, with 73%5 to 81.9% entrapment efficiency and 14.79% to 16.38% drug loading in the nanoparticles. The polymer concentration was found to influence the % entrapment efficiency and release characteristics of nanoparticles.

The *in-vitro* drug release studies by using dialysis membrane 150 showed that after the initial burst, all the different drug-loaded CBN provided a continuous and slow release of the drug. The CBN system demonstrates capability to almotriptan, antimigraine drug to potentially provide an effective treatment option in migraine therapy. *In-vit*ro kinetic studies was also performed and it indicates a lesser amount of linearity when plotted by the zero-order equation. Hence, it can be concluded that the major mechanism of drug release follows first order kinetics.

Polymer and drug was found to be compatible from DSC studies and powder x-ray diffraction studies. Mucoadhesion studies were also conducted using goat nasal mucosa and mucoadhesion percentage was between 54% and 69%. It was concluded that mucoadhesive nanoparticles as intranasal formulation is an alternative drug delivery system for nose to brain transport. From the stability studies it was observed that there is not significant changes in the physical property at room temperature and 5 ± 1°C.

Present investigation, hence, led to successful nose to brain targeting of the hydrophilic drug almotriptan malate by its incorporation in chitosan biodegradable nanoparticles for the efficient therapeutic management of migraine. The future perspective of this project is that *in-vivo* studies should be done in this the optimised formulation and the optimized formulation can be combined with the free drug along with the penetration enhancers to enhance the release of the drug to provide quick action and prolonged release to treat mild to moderate migraine. All results of this research work provide useful information for future studies aiming at development of drug delivery formulation consisting of chitosan nanoparticles and with drug almotriptan malate for treatment of migraine.

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